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Reaction Centers from *Rhodopseudomonas sphaeroides* In Reconstituted Phospholipid Vesicles. II. Light-Induced Proton Translocation[1]

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Abstract

Unidirectional light-dependent proton translocation was demonstrated in a suspension of reconstituted reaction center (RC) vesicles supplemented with cytochrome *c* and 2,3-dimethoxy-5-methyl-1,4-benzoquinone (UQ_0), a lipid- and water-soluble quinone. Proton translocation was detected only at alkaline pH. The pH dependence can be accounted for by the slow redox reaction between the reduced quinone (UQ_0H_2) and oxidized cytochrome *c*. This conclusion is based on (i) the pH dependence of partial reactions of the reconstituted proton translocation cycle, measured either optically or electrometrically and (ii) titration studies with cytochrome *c* and UQ_0. At 250 and 25 μM UQ_0 and cytochrome *c*, respectively, maximal proton translocation was observed at pH 9.6. This pH optimum can be extended to a more acidic pH by increasing the concentration of the soluble redox mediators in the reconstituted cyclic electron transfer chain. At the alkaline side of the pH optimum, proton translocation appears to be limited by electron transfer from the endogenous primary to the secondary quinone within the RCs. The light intensity limits the reconstituted proton pump at the optimal pH. The results are discussed in the context of a reaction scheme for the cyclic redox reactions and the associated proton translocation events.

[1] Abbreviations: RC: reaction center; UQ_0/UQ_0H_2: oxidized and reduced form of 2,3-dimethoxy-5-methyl-1,4-benzoquinone; D/D^+: reduced and oxidized form of the primary electron donor of the RCs; CCCP: carbonylcyanide-trichloromethoxy phenylhydrazone; UQ_A/UQ_A^-: oxidized and semiquinone form of the primary electron acceptor of the RCs; $UQ_B/UQ_B^-/UQ_BH_2$: oxidized, semiquinone, and reduced form of the secondary electron acceptor of the RCs; LDAO: lauryldimethylamine-*N*-oxide.

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Introduction

The primary reaction catalyzed by reaction centers (RCs) *in vivo* is the transduction of light energy into an electrical potential- and redox energy-gradient across the energy-transducing cytoplasmic membrane (Feher and Okamura, 1978; Parson, 1982; Dutton *et al.*, 1982). The molecular basis of this primary event is the transfer of an excited electron from the primary electron donor of the RCs (D or P870, a special bacteriochlorophyll dimer) to the primary or secondary electron acceptor (both of which are ubiquinone molecules, usually abbreviated as UQ_A and UQ_B, respectively). *In vivo*, charge recombination proceeds via a complex series of redox reactions in a proton-translocating bacterial cytochrome b–c_1 complex (Hauska *et al.*, 1983) and via cytochrome c_2. The result of light absorption then is the generation of a protonmotive force (Mitchell, 1968), with a stoichiometry of two protons per cycled electron (Dutton *et al.*, 1982). The protonmotive force drives a variety of cellular energy requiring processes (Hellingwerf and Konings, 1985).

A major question in electron transfer within the RCs is the influence of imposed energy gradients (of electrical potential and redox energy) on the rates of electron transfer. These aspects were explored in intact cells or isolated bacterial cytoplasmic membranes, where many complex processes proceed simultaneously (Jackson *et al.*, 1981; Cotton and Jackson, 1982).

A more direct approach would be to study the transmembrane events associated with the photochemical reactions in a reconstituted system that is amenable to experimental control (Montal *et al.*, 1981). Reconstituted RC vesicles in which unidirectional proton translocation generates a protonmotive force would provide a system where its effect on the rate(s) of electron transfer within the RCs can be investigated. Proton translocation in reconstituted RC vesicles (Crofts *et al.*, 1977; Darszon *et al.*, 1980) was observed only in the presence of lipid-soluble redox mediators.

In the preceding paper, the preparation and structural properties of reconstituted RC vesicles were described. Here, a detailed investigation of the light-induced proton translocation events is presented. A preliminary account of this work has been presented (Hellingwerf, 1984).

Experimental

Materials

Horse heart cytochrome *c* and valinomycin were from Sigma Chemicals, St. Louis, Missouri; nigericin was kindly supplied by H. E. Holden from

Eli Lilly and Co., Indianapolis, Indiana; 2,3-dimethoxy-5-methyl-1,4-benzoquinone (UQ_0) was from Trans World Chemicals, Washington, DC, and all other materials were as described in Part I of this paper (Hellingwerf, 1987). The UQ_0 was used as a stock solution in ethanol (100 mM), stored at 4°C, and was about 1–3% reduced.

Proton Translocation Measurements

Proton translocation was measured as the change in pH of the external medium of a suspension of reconstituted RC vesicles upon illumination. The instrument consisted of an O-I pH 2000 combination electrode (Owens-Illinois, Inc., Toledo, Ohio) connected to a Model 600B Keithley electrometer (Keithley Instruments, Inc., Cleveland, Ohio). The output signal from the electrometer was continuously recorded on a strip chart recorder (Hewlett-Packard, Model 7035B X–Y recorder). The flat-bottomed pH electrode (diameter 14 mm) was inserted sideways into a thermostated chamber, constructed by drilling a 16-mm-diameter hole in a Lucite block. The chamber had an adjustable working volume between 1.0 and 4.0 ml. It was mounted on a magnetic stirrer (Vanlab magnetic stirrer/hot plate, VWR Scientific Inc.) for mechanical support and for mixing. It was thermostated at 30°C by means of a circulating water bath. The chamber was covered with a Teflon stopper or a cover glass and was flushed with water-saturated argon to obtain anaerobiosis. Light was supplied by an Ealing Model 22-0004 fiber light source (The Ealing Corp., South Natick, Massachusetts), equipped with a 150-W tungsten filament lamp. The light was passed through a 10-cm water filter which also focused the light on a mirror which in turn reflected the actinic light on the chamber. In addition, the light was filtered through a long-pass cut-off filter (50% transmission at 660 nm). With this arrangement, a light intensity of 40 mW/cm^2 (measured with an YSI-Kettering No. 65 radiometer) was obtained at the surface of the chamber. The pH of the suspension was read directly from the Keithley electrometer, after appropriate calibration of the system with regular pH calibration buffers between pH 4 and 10 (Scientific Products, McGaw Park, Illinois; Fisher Scientific Co., Fair Lawn, New York, and Mallinckrodt Inc., Paris, Kentucky). The pH changes in the chamber upon illumination were calibrated with the addition of known quantities of oxalic acid. Due to the geometry of the chamber, high stirring speed ($\sim$ 50 Hz) is required for a rapid response of the electrode to pH changes; the response time was $\sim$ 1 s. The pH measurements were routinely performed after the successive addition to the chamber of 2.0 ml anaerobic 200 mM KCl, 50 μl 1 mM reduced cytochrome *c*, 5 μl 100 mM UQ_0 (partially reduced, see Materials), $\sim$ 50 μl of a suspension of RC vesicles reconstituted by sonication (typically 0.1–0.5 mg RC protein) and 5 μl 1 mM valinomycin. Proton translocation measurements were performed in the

presence of a saturating amount of valinomycin (i.e., 2.5 μM), which in 200 mM KCl collapses the transmembrane electrical potential thereby simplifying the interpretation of the measurements (Hellingwerf *et al.*, 1978). RC vesicles prepared by cholate dialysis were assayed in th same range of protein concentrations (Hellingwerf, 1987). When ethanolic solutions were added (cf. valinomycin, UQ_0, *o*-phenanthroline, etc.), the total ethanol content of the sample was kept below 1% (v/v). Preceding the actual measurements, the mixture was equilibrated for 20 min in the dark in the vessel for pH measurements to reduce the initial drift in the pH of the sample. Changes in pH as small as 10^{-4} pH unit ($\sim$ 1 nmol protons) are detected with this instrument. Measurements of the initial rate and extent of proton translocation on each sample were performed in replicate.

All other methods used were as described in Part I of this paper (Hellingwerf, 1987).

Results

Light-Induced Proton Translocation by Reconstituted RCs

Figure 1 illustrates the sequence of electron transfer reactions and associated proton translocation events considered to proceed upon illumination of reconstituted RCs supplemented with cytochrome *c* and UQ_0. Accordingly, upon illumination of a suspension of RC vesicles, a cyclic electron transfer chain would operate and produce a unidirectional proton translocation (Fig. 1A). The electrogenic nature of such a pump arises from the charge separation event in the RC, whereas proton translocation is accounted for by the transmembrane diffusion of the reduced (UQ_0H_2) and oxidized (UQ_0) forms of ubiquinone in opposite directions. The main difference between this reconstituted proton pump and the cyclic electron transfer chain operating *in vivo* is the proton-to-electron stoichiometry, which is 1 (Fig. 1A) and 2 (Dutton *et al.*, 1982), respectively. A detailed representation of the redox reactions involved in the reconstituted cyclic electron transfer chain is given in Fig. 1B. At least five redox species can be distinguished, which undergo oxidation/reduction transitions during illumination. These couples are: the primary RC electron donor (D^+/D); the primary RC electron acceptor (UQ_A/UQ_A^-); the secondary RC quinone, which participates in three forms ($UQ_B/UQ_B^-/UQ_BH_2$); the water- and membrane-soluble exogenous quinone (UQ_0/UQ_0H_2); and the reduced and oxidized forms of cytochrome *c*, an exogenous electron donor.

The protonation state of most of these components depends on their redox state(s). This generates light-induced pH changes in a suspension

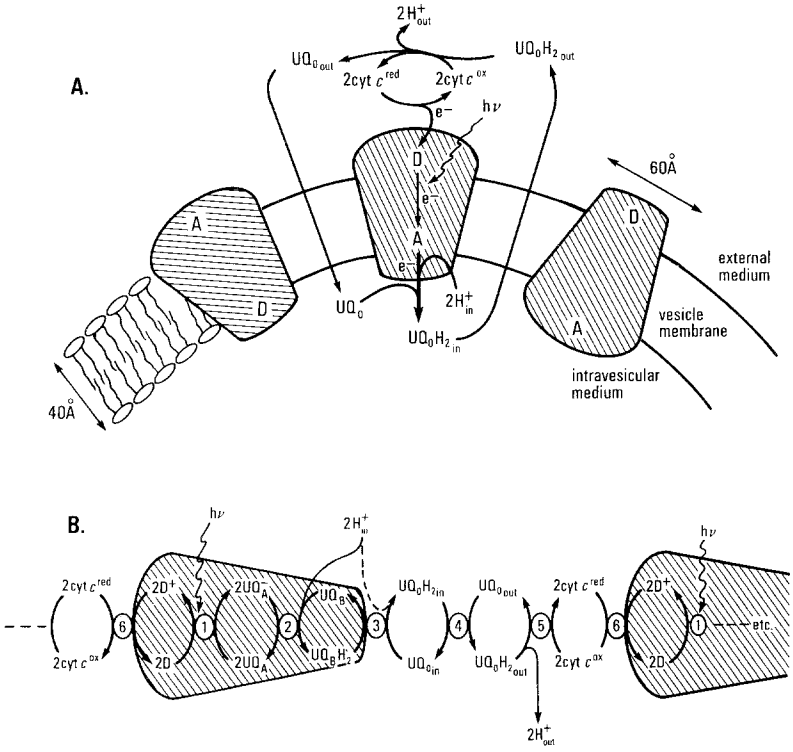

Fig. 1. Schematic representation of the cyclic chain of redox reactions involved in proton translocation by reconstituted RC vesicles. (A) Topological scheme of electron and proton translocation reactions. (B) Detailed scheme of the participating sequential redox reactions. The reactions illustrated in the shaded area occur within the RCs. The assignment of UQ_B as the component where the proton enters the translocation cycle is tentative (Kleinfeld, D., personal communication). The subscripts in and out refer to the presence of the particular component in the intravesicular or extravesicular medium, respectively.

of RC vesicles, even if the permeability barrier of the membrane for protons is abolished by the addition of a protonophore or is completely removed by detergent solubilization. Figure 2A shows the complex pattern of pH changes that is observed in a proton translocation assay of reconstituted RC vesicles at pH 8.5. At this pH, illumination evokes first a transient alkalinization followed by an acidification (trace a). The relative magnitude of the two phases is pH dependent and the transient alkalinization disappears at high pH (> 9.5). The use of ionophores in this assay allows one to identify the alkalinization as arising from scalar pH changes associated with the redox state of components of the reconstituted electron transfer system, and the acidification arising from the pH changes generated by vectorial proton translocation, respectively. The acidification is sensitive to protonophores,

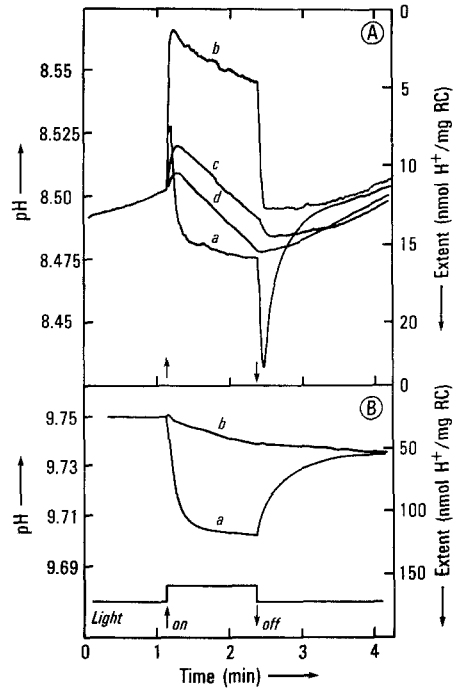

Fig. 2. Light-dependent pH changes in a suspension of RC vesicles, supplemented with redox mediators. (A) pH 8.5; (B) 9.8. Vesicles were reconstituted from LDAO-depleted RCs and asolectin (molar ratio 1:560) by sonication; 50 μl of this suspension was incubated in the chamber for pH measurements, together with 2 ml 200 mM KCl, 50 μl 1 mM reduced cytochrome *c*, 5 μl 100 mM UQ_0, and 5 μl 1 mM valinomycin. The pH of the suspension was adjusted with 200 mM KOH or 100 mM oxalic acid. Light was turned on and off as indicated. (A) pH 8.5. a: no additions; b: plus 5 μl 10 mM CCCP; c, d: plus 5 and 10 μl 3 M *o*-phenanthroline, respectively. (B) pH 9.8. a: no additions; b: plus 5 μl 10 mM CCCP. The pH changes were calibrated with 1 mM oxalic acid.

carbonylcyanide-trichloromethoxyphenylhydrazone (CCCP), and pH-gradient dissipating ionophores, nigericin (trace b of Fig. 2A). The alkalinization is sensitive to *o*-phenanthroline, an inhibitor of electron transfer between the primary and secondary quinone of the RCs (trace c and d in Fig. 2A) and, therefore, is accounted for by the shift in the redox state of cytochrome *c* and UQ_0 upon illumination.

At pH 9.8 (Fig. 2B) the transient alkalinization is absent. The pH changes exhibit a time course characteristic of an outwardly directed proton pump. The pH dependence for proton translocation in a suspension of RC vesicles was determined. Figure 3 shows that both the initial rate and the extent of the proton extrusion are distinctly pH dependent with an optimum between pH 9.5 and 9.75 in the presence of exogenous redox mediators.

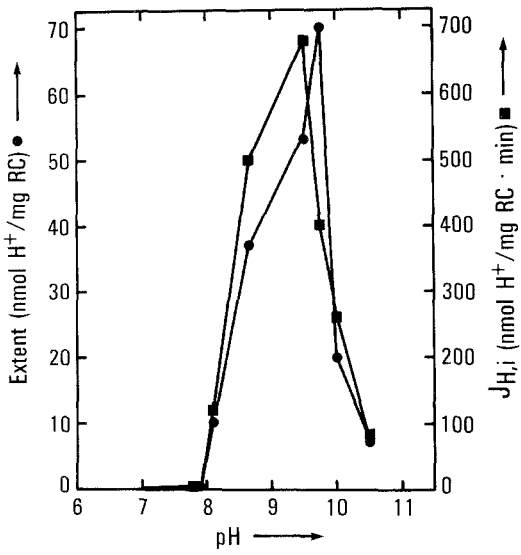

Fig. 3. pH Dependence of proton translocation in RC vesicles. The RC vesicles were prepared by cholate dialysis at a molar lipid/RC ratio of 560. Extent: magnitude of the pH change in the extravesicular medium upon illumination, in nmol H^+/mg RC. $J_{H,i}$: initial rate of light-dependent proton extrusion. Other conditions are as described in Fig. 2. Where necessary (pH < 9.8), the initial rate of proton translocation was measured from the difference between an experiment with and an experiment without 24 μM CCCP.

Characterization of the Reconstituted Proton Pump

To gain further insight into the variables that govern proton extrusion catalyzed by reconstituted RC vesicles, especially with respect to the parameters that limit the turnover rate of the reconstituted proton pump, the experiments described in Figs. 4–6 and in Table I were performed. The dependence of the reconstituted proton pump activity on the concentration of UQ_0 is illustrated in Fig. 4. At pH 9.9 (panel B), proton translocation saturated at 100 μM UQ_0. The upper panel of Fig. 4 shows that at pH 8.5 higher UQ_0 concentrations were required to saturate the extent of proton translocation: half-saturation was obtained at $[UQ_0] = 200\,\mu M$. This UQ_0 titration is associated with gradual changes in the waveform of the light-induced proton translocation from that illustrated in Fig. 2A to that shown in Fig. 2B, where the transient initial alkalinization disappeared. Thus, increasing the ubiquinone concentration during reconstitution effectively extends the pH range in which the activity of the light-driven proton pump is measurable (at the acidic side of the pH optimum, compare Fig. 3). The open circles in Figure 4A represent the magnitude of the light-induced alkalinization and the closed circles represent the uncoupler sensitive

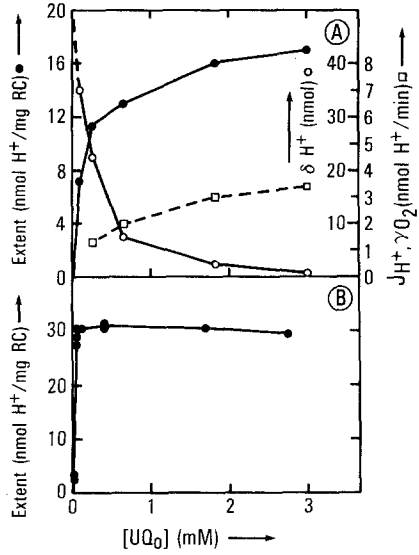

Fig. 4. Dependence of the light-induced proton translocation in RC vesicles on the concentration of quinone. (A) pH 8.5; (B) pH 9.9. (A) RC vesicles were prepared from asolectin and LDAO-depleted RCs (in a molar ratio of 560) by sonication. Light-dependent pH changes were measured as described in Methods at pH 8.5, except that the UQ_0 concentration was varied. δH^+: extent of the uncoupler insensitive alkalinization upon illumination. $J_{H^+,\gamma O_2}$: rate of the uncoupler and *o*-phenanthroline-insensitive, light-dependent acidification. (B) RC vesicles were prepared by cholate dialysis at the same lipid/RC ratio as in A. Light-dependent proton translocation was measured at pH 9.9 at the indicated UQ_0 concentrations.

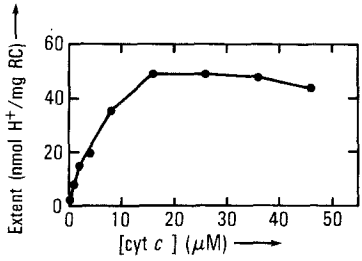

Fig. 5. Dependence of the light-induced proton translocation in RC vesicles on the concentration of cytochrome *c*. Vesicles were prepared as described in the legend to Fig. 4, part B, and the UQ_0 concentration was fixed at 250 μM.

fraction of the signal, i.e., the extent of proton translocation. The open squares in Fig. 4A represent the rate of the light-induced but uncoupler and *o*-phenanthroline insensitive acidification. This rate increases with the quinone concentration and is strongly enhanced by the omission of argon during the assay. Therefore, this signal can be accounted for by the photooxidation of a reduced form of ubiquinone.

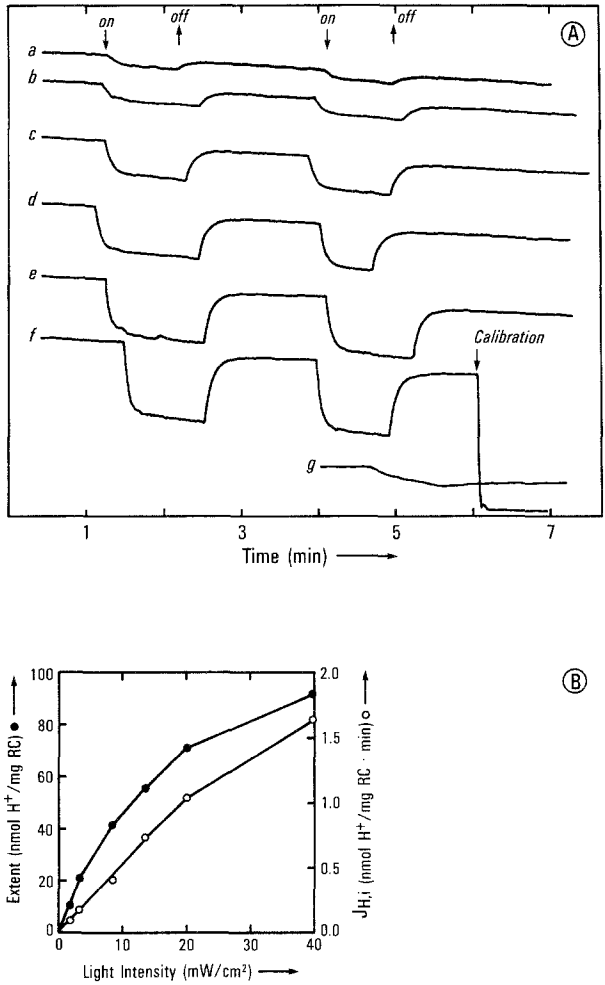

Fig. 6. Light intensity dependence of the initial rate and extent of proton translocation in reconstituted RC vesicles. (A) Recordings of the experimental observations. (B) Light intensity dependence of the initial rate and extent of proton extrusion. (A) RC vesicles were reconstituted from asolectin and LDAO-depleted RCs (molar ratio 560) by sonication for 30 min. Proton translocation was assayed at pH 9.75. The light intensity was varied with neutral density filters to 4.5, 8, 21, 34, 50, and 100% of the maximal intensity (40 mW/cm^2), respectively. At each intensity the light was turned on and off twice for periods of approximately 1 and 2 min, respectively, as indicated. pH changes in the suspension were calibrated with 20 nmol H^+ as indicated; the resulting pH change corresponds to 0.033 pH units. In trace (g) 3.3 μl 3 M *o*-phenanthroline was added. The traces are contiguous segments of approximately 7 min from a continuous record. Drift in pH during this 1 h experiment was less than 0.05 pH units. (B) Double reciprocal plot of the data from (A).

Table I. pH Dependence of the Reduction of Cytochrome *c* by Reduced UQ_0 (UQ_0H_2), as Measured by Three Different Methods.[a]

pH	A	B	C
6		> 1000	n.d.
7	400	100	660
8	5	4	8
9	< 1	< 2	< 1
10	< 1	n.d.	n.d.

[a]The numbers represent the half-times of the reaction in seconds. n.d.: not determined. A: oxidized cytochrome *c* (25 μM) was reduced with partially reduced UQ_0 (250 uM, 1–3% reduced, see Materials). B: The half-time of the decay of the light-dependent uncoupler-insensitive alkalinization was measured in the indicated pH range as described in the legend to Fig. 2, in the presence of 5 μl 10 mM CCCP. C: subsequent to photooxidation, the rereduction of cytochrome *c* was measured at 550 nm, under conditions that mimicked those of proton translocation measurements, except that 1 ml pH calibration buffer was added to adjust the pH.

Figure 5 shows the dependence of the light-induced proton translocation on cytochrome *c*. The maximal extent was obtained at the cytochrome *c* concentration that was routinely used in the assays, namely 25 μM. Under these conditions (200 mM KCl) a K_m for cytochrome *c* of 4.4 μM is calculated. At high cytochrome *c* concentrations ($\geq$ 30 μM) a slight decrease in the extent of proton translocation was observed. This may arise from aggregation of RC vesicles that bear the negatively charged lipids of asolectin by the positively charged cytochrome *c*.

Table I shows the kinetic relationship between the rate of alkalinization and the rate of cytochrome *c* reduction by reduced UQ_0. This confirms that the rapid and uncoupler-insensitive alkalinization observed upon illumination is due to a change in the protonation state of a redox intermediate in the reconstituted proton pump. The first method (column A) measures directly the chemical reaction between reduced UQ_0 and oxidized cytochrome *c*: The reaction rate increased with pH, presumably due to the involvement of the semiquinone form of UQ_0 (in agreement with Hurt and Hauska, 1981). In column B, the decay half-time of the uncoupler-insensitive alkalinization, measured electrometrically, is tabulated. The results indicate that, in the presence of an uncoupler, the effect of light is the oxidation of cytochrome *c* by the RCs and the subsequent transfer of reducing equivalents to UQ_0. These electrons are presumably neutralized by protons to form UQ_0H_2 causing the rapid, uncoupler-insensitive alkalinization as illustrated in Fig. 2A. Column C shows the reaction rate between oxidized cytochrome *c* and reduced UQ_0, but here cytochrome *c* was photooxidized by a suspension of the reconstituted RCs. The results indicate that as the pH is lowered, the rereduction of photooxidized cytochrome *c* by UQ_0H_2 becomes increasingly rate limiting. At low pH (< 7.5) cytochrome *c* is photooxidized completely,

and at higher pH (> 9.5) it is fully reduced (compare also Figs. 2A and 2B). The pH at which this transition is 50% equals 8.3. The same pH value is obtained for the half-maximal transition in redox state of the primary electron donor of the RCs.

Light Intensity Dependence of the Reconstituted Proton Pump

The light intensity dependence of the reconstituted proton pump is illustrated in Fig. 6. In A, direct recordings of the pH changes are reproduced, to indicate the stability and accuracy of the experimental system. Anaerobiosis of the pH-meter vessel and a battery-powdered pH meter are critical in this respect. In B, the dependence of the initial rate and extent of proton translocation on light intensity is plotted. From this figure it is clear that the degree of light saturation of the extent of proton translocation is larger than for the initial rate of proton translocation. From a double reciprocal plot of these data (not shown), K_m's of $20\,mW/cm^2$ for the extent and $\pm 100\,mW/cm^2$ for the initial rate of proton translocation can be calculated. These data furthermore show that the degree of light saturation of the extent of proton translocation is 69% and for the initial rate of proton translocation approximately 30%.

Discussion

In these studies on proton translocation in RC vesicles no elementary differences between vesicles, prepared by sonication or dialysis, have emerged, neither in the structural nor in the functional characterization. As is evident from Part I of this paper (Hellingwerf, 1987) the most notable differences in structure are caused by the presence or absence of LDAO, not by the use of sonication or dialyses. The procedure for removal of LDAO in the latter two methods is very similar. A similar conclusion applies to reconstituted bacteriorhodopsin liposomes (Hellingwerf, 1979).

The unidirectionality of proton translocation by the reconstituted proton pump would be lost if the rereduction rate of D^+ directly via reduced UQ_0 were significant. However, from the results of Fig. 5 it is clear that under conditions when 70% of the RCs are oriented with their cytochrome *c* binding site toward the exterior of the vesicles (i.e., a 40% effective net orientation), in the absence of cytochrome *c*, the extent of proton translocation is negligible. This implies that the rate of reduction of D^+ by reduced UQ_0 can be neglected in comparison with the rate of reduction of D^+ by reduced cytochrome *c* and thus that the reconstituted proton pump is essentially unidirectional.

The reconstituted proton pump operates in a narrow pH range under the standard conditions of 250 μM UQ_0 and 25 μM cytochrome *c*, although this range can be broadened by an increase in the UQ_0 concentration (Fig. 4). The reactions that limit the reconstituted proton pump are critically dependent on pH. At low pH (< 8) the rereduction of cytochrome *c* by reduced UQ_0 is rate limiting. This conclusion is based both on direct measurement of the reaction (Table I) and on titration studies with UQ_0 (Fig. 4). At the optimal pH (~ 10) the light intensity limits the rate of proton pumping (Fig. 6). The maximal rate of proton translocation that can be calculated from the experiment described in Fig. 3, corrected for the nonsaturating intensity of the actinic illumination (Fig. 6) and the partial randomization of the RCs (Hellingwerf, 1987), equals $7\,s^{-1}$. At high pH (> 10), the reconstituted proton pump appears to be limited by the electron transfer between the primary and secondary quinones of the reaction centers (reaction 2 in Fig. 1). This is based on the following information: (i) The titration studies in Figs. 4B and 5 show that the limitation is not in the reactions in which the soluble redox mediators UQ_0 and cytochrome *c* are involved. The relatively high K_m of the RCs for cytochrome *c* is most likely due to the relatively high ionic strength used (200 mM KCl; Rosen *et al.*, 1981). (ii) The rate of the reaction of the second electron from the primary to the secondary quinone is the slowest of the redox reactions taking place in the reconstituted proton pump (Kleinfeld *et al.*, 1984, 1985), particularly of the electron transfer reactions within the RCs. This rate decreases from $10\,s^{-1}$ at pH 10.1 to below $1\,s^{-1}$ at pH 11 (Kleinfeld *et al.*, 1985), thereby parallelling the rate of proton translocation in this pH range. (iii) At high pH the oxidized primary electron donor of the RCs, generated by a flash, shows the same decay time as obtained at low pH after inhibition of electron transfer with *o*-phenanthroline (data not shown).

The results presented in Fig. 6 show a distinct difference between the degree of light saturation of the initial rate and extent of proton translocation. This, and the acceleration of the rate of proton translocation induced by valinomycin, are evidence for "back pressure" of the protonmotive force on the rate of proton translocation by reconstituted RC vesicles, comparable to the situation in bacteriorhodopsin liposomes (Westerhoff *et al.*, 1979, 1981; Hellingwerf *et al.*, 1979). In studies of RCs in planar lipid bilayers the effect of an electric field on electron transfer in the RCs was reported (Gopher *et al.*, 1983).

In vesicles that contain an RC-based proton pump there is a fair agreement between the rate-limiting step of electron transfer in the reconstituted proton pump and the rate of proton translocation. In contrast, for the proton pump of bacteriorhodopsin, a large discrepancy exists between the maximal rate of proton translocation and the rate-limiting step(s) in the photocycle of this retinal-based proton pump (Hellingwerf, 1979).

Reconstitution of proton translocation with isolated RCs was reported with Photosystem II RCs from chloroplasts (Orlich and Hauska, 1980; Hauska *et al.*, 1980). This study focused on the coreconstitution of RCs and an ATPase complex to form vesicles active in photophosphorylation. The strong pH dependence of the reaction between reduced quinones and oxidized cytochrome *c* was also reported (Hurt and Hauska, 1981).

The reconstituted vesicles described in this report show a high leakiness toward ascorbic acid and/or ferricyanide, rendering the assay of RC orientation complex (see Casey *et al.*, 1982). This prohibits a simple assay for "back-pressure" in the RC proton (or electron) pump. The RC vesicles described are a starting point for the reconstitution of a proton-translocating cyclic electron transfer chain from isolated RCs and a cytochrome b/c_1 complex (Packham *et al.*, 1980), for photophosphorylation with the coreconstitution of RCs and an ATPase complex, and for fusion experiments between biological membranes and reconstituted membranes containing a proton pump (Driessen *et al.*, 1985).

Conclusions

The requirements for unidirectional proton translocation in a suspension of RC vesicles, supplemented with cytochrome *c* and UQ_0, were described. Under some conditions the extravesicular pH changes evoked by illumination are dominated by scalar pH changes associated to shifts in the redox state of cytochrome *c* and UQ_0, and not to pH changes due to vectorial proton translocation. The reactions that restrict the rate of the reconstituted proton pump were partially resolved, assayed, and identified.

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