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LIGHT-ACTIVATED PROTON-MOTIVE FORCE GENERATION IN LIPID VESICLES CONTAINING CYTOCHROME b-c_1 COMPLEX AND BACTERIAL REACTION CENTRES

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(i) Purified bovine heart mitochondrial cytochrome b-c_1 complex (ubiquinone-cytochrome c oxidoreductase) and photosynthetic reaction centres isolated from *Rhodopseudomonas sphaeroides* strain R-26 have been incorporated into lipid vesicles. In the presence of cytochrome c and ubiquinone-2, light activation caused a cyclic electron transfer involving both components. (2) Since cytochrome c is added outside the vesicles, it is both reduced by the cytochrome b-c_1 complex and oxidised by the reaction centre on the outside of the vesicles. Ubiquinone-2, however, is reduced by the reaction centres at a site in contact with the inside of the vesicles, but the reduced form, ubiquinol-2, is oxidised by the cytochrome b-c_1 complex at a site in contact with the outer aqueous phase. (3) In the presence of valinomycin plus K^+, initiation of cyclic electron flow causes protons to move from inside the vesicles to the outer medium and the $H^+/2e^-$ ratio was calculated to be close to 4.

Introduction

The reincorporation of bioenergetic protein components into lipid vesicles has been widely achieved. Reincorporation of cytochrome b-c_1 complex [1–3], cytochrome b-f complex [4], ATPase [5], cytochrome oxidase [6,7], bacteriorhodopsin [8] and bacterial reaction centres [9,10] has all produced systems which can generate a proton-motive force across the membrane when an appropriate energy source is provided.

This report describes the reincorporation of a hybrid system of mammalian cytochrome b-c_1 complex and reaction centres isolated from a carotenoidless mutant of *Rhodopseudomonas sphaeroides* into soya bean phospholipid vesicles. In solution, such a combination has already been shown to act as a cyclic electron-transport system [11,12]. In the vesicles, with cytochrome c and ubiquinone-2 added as redox connectors, light activation causes cyclic electron transport and concomitant generation of a proton-motive force across the vesicle membranes. By measurement of rate constants and extents of the component reactions, it may be shown that the $H^+/2e^-$ ratio is initially 4. Evidence is presented to show that two protons are moved across the membrane by the vectorial arrangement of the reaction sites, whereas the extra protons are moved by an additional proton-motive ability of the cytochrome b-c_1 complex.

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Abbreviations: Tricine, *N*-tris(hydroxymethyl)methylglycine; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

Methods

Preparation of protein components

Cytochrome b-c_1 complex was prepared from bovine heart mitochondria by the method of Hatefi and Rieske [13,14]. It was stored at 77 K at a cytochrome c_1 concentration of 100–150 μM in 5 mM K^+ Tricine at pH 8.0. Reaction centres were prepared by lauryldimethylamine N-oxide treatment of chromatophores from the carotenoidless mutant R-26 of *Rps. sphaeroides* and purified by ammonium sulphate fractionation as previously described [15]. Cytochrome c was purchased from Sigma (horse heart, type VI).

Electron-transfer rate measurements

Electron-transfer rates were monitored by measurement of the rates of redox state change of cytochrome c at 550 nm and using an $\epsilon_{mM^{-1}\cdot cm^{-1}}$ of 19. A Cary model 15 spectrophotometer was used for routine assays of catalytic activity from added quinol to ferricytochrome c. More quantitative changes and their analyses were performed with an Applied Photophysics single-beam instrument interfaced to an Apple II microcomputer capable of kinetic analyses of decay curves.

Measurement of proton-extrusion rates

The rates of protons appearing in the external medium were assayed with phenol red as a pH-sensitive indicator. The medium used was 160 mM sucrose, 40 mM KCl and 0.5 mM EDTA, generally at pH 7, and containing 1 mM KCN, 0.1 μg/ml valinomycin and 100 μM phenol red. Changes in protonation state of phenol red were monitored at 542 nm, an isosbestic point for cytochrome c spectral changes. In practice, the monochromator was finely tuned around this wavelength until cytochrome c redox changes had no effect on spectral changes. Calibration was achieved with aliquots of standard HCl or KOH, or by other internal standards available in most of the experiments (see text).

Illumination

This was provided by a 12 V, 150 W projector lamp (General Electric BLV, 081) with a Schott glass RG 715 filter to cut off light below 715 nm. The photomultiplier was protected by a combination of a Wratten green filter plus a 1 cm saturated $CuSO_4$ solution, together with a short pass filter with a cut-off at 605 nm.

Other chemicals

Ubiquinone-2 was the kind gift of Hoffmann-La Roche, Basel, Switzerland, DBMIB of Professor A. Trebst and myxothiazol of Dr. W. Trowitzch. Duroquinol and ubiquinol-2 were prepared from the quinones as previously described [16].

Reincorporation into lipid vesicles

The technique followed was the cholate dialysis technique [17,18]. Firstly, soya bean asolectin (Sigma, type IV-S) was washed with acetone, dried with nitrogen and redissolved in chloroform. The lipid was then dried onto the surface of a round-bottomed flask by evaporation with nitrogen. Buffer (0.1 M potassium phosphate, pH 7.8) was then added, together with purified potassium cholate. The ratio of components used was 30 mg lipid:15 mg cholate:1 ml buffer. The whole was vortex mixed until all lipid had left the sides of the vessel and a cloudy solution had formed. This was sonicated at 0°C in a water bath sonicator until translucent. Cytochrome b-c_1 complex (2.5 nmol/ml vesicles) and reaction centres (2.5 nmol/ml vesicles) were then added. The resulting mixture was dialysed for 5 h against 0.1 M potassium phosphate at pH 7.8, and then overnight against 0.16 M sucrose, 40 mM KCl and 0.5 mM EDTA at pH 7. Such a sequence of dialyses ensured a high buffering capacity (0.1 M potassium phosphate) inside the vesicles. The resulting vesicle suspension was translucent and was kept out of bright light until used.

Results

'Respiratory control' in the hybrid vesicle system

In order to test routinely whether proteins had reincorporated into reformed vesicles, an assay was performed of the effect of uncoupler on the cytochrome b-c_1 complex-catalysed reaction of quinol reduction of ferricytochrome c [1,2,19]. Such an assay is illustrated in Fig. 1, in this case with duroquinol used as the quinol reductant. Stimulation of the enzymic quinol-cytochrome c oxidoreductase activity indicated that reincorporation into

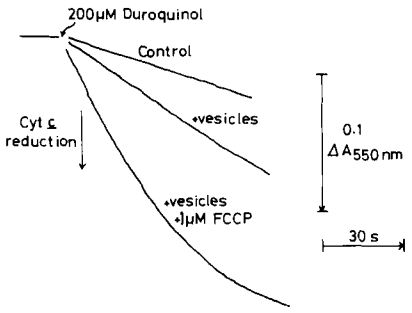

Fig. 1. Respiratory control in cytochrome b-c_1 complex reaction centre vesicles. Reaction medium was 160 mM sucrose, 40 mM KCl and 0.5 mM EDTA at pH 7.0 and 25°C. Other additions were 1 mM KCN, 11 μM ferricytochrome c and vesicles to a concentration such that cytochrome b-c_1 complex was 8 nM. The reaction was started by addition of 200 μM duroquinol and the reduction of cytochrome c was monitored at 550 nm. In this particular experiment, after subtraction of the non-enzymatic control rate, an uncoupler stimulation of 4.5-fold is seen with an uncoupled turnover number of the enzyme of 25 s^{-1}.

vesicles had occurred. Typical values of uncoupler stimulation of the enzymic rate (after subtraction of the basal chemical rate) were between 2- and 5-fold, when 200 μM duroquinol and 11 μM ferricytochrome c were used. The effects of changes in reagent concentrations on observed respiratory control in the cytochrome b-c_1 complex vesicle system have already been discussed [19]. Similar results could be obtained with ubiquinol-1 or ubiquinol-2 as donors in place of duroquinol.

Light-activated proton pumping

Fig. 2 illustrates proton-pumping activity of the vesicle system on light activation. In the presence of cytochrome c alone or ubiquinone-2 alone significant pumping occurred, but was most extensive in the presence of both together. In the absence of valinomycin to remove any large $\Delta\psi$ build-up [20], the extent of pumping observed was small and slow (Fig. 2), hence demonstrating the electrogenic nature of the pumping process.

The overall profile of the light-activated pumping process was complex. Commercial cytochrome c was about 4–6% reduced as purchased and hence in the type of experiment shown in Figs. 2 and 3, some electrons were already present in the system which before light activation will be located on the cytochrome c. On light activation, however, the ferrocytochrome c is rapidly oxidised while an equivalent amount of ubiquinone-2 is reduced, causing a net removal of protons from the reaction

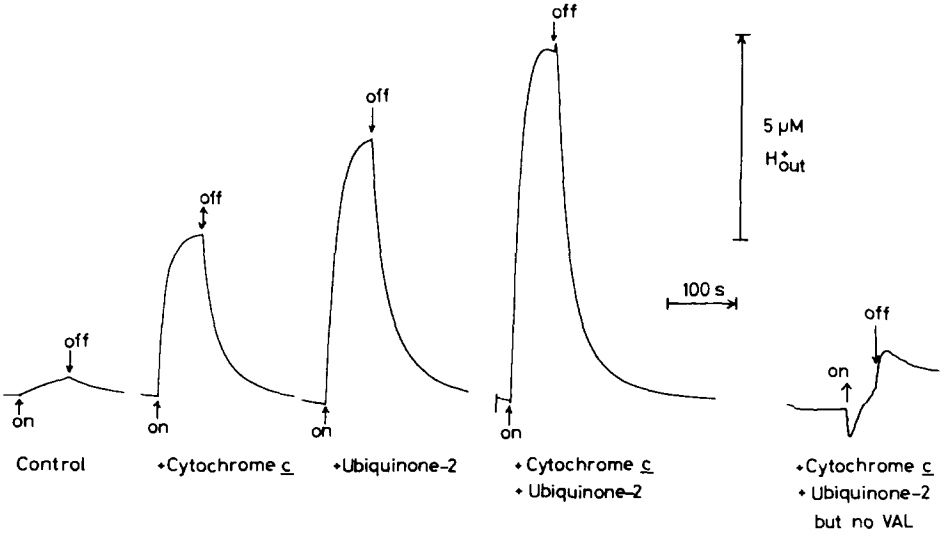

Fig. 2. Proton pumping in cytochrome b-c_1 complex reaction centre vesicles. Reaction medium was 160 mM sucrose, 40 mM KCl and 0.5 mM EDTA at pH 7.2 and 23°C. Other additions were 1 mM KCN, 0.1 μg/ml valinomycin (VAL), and 100 μM phenol red and vesicles to a final cytochrome b-c_1 complex concentration of 53 nM. The appearance of protons in the external medium was monitored by a decreased absorbance at 542 nm (upward deflection) and was calibrated with aliquots of standard HCl. Arrows indicate times of switching light on and off.

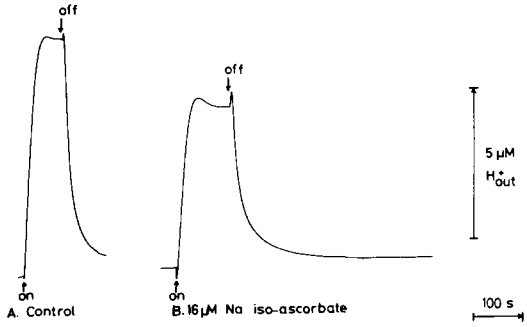

Fig. 3. The pool effect on the proton-pumping profile of cytochrome b-c_1 complex reaction centre vesicles. Conditions were the same as in Fig. 2 with 14.5 μM cytochrome c and 4 μM ubiquinone-2 present in both. H^+_{out} was monitored at 542 nm with an upward deflection representing an increase in H^+_{out}. In trace A the cytochrome c was 5% reduced whereas in trace B the cytochrome c was more than 95% reduced by the added sodium isoascorbate.

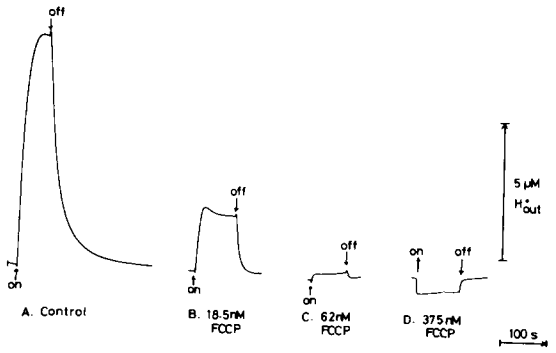

Fig. 4. Uncoupler sensitivity of proton pumping in cytochrome b-c_1 complex reaction centre vesicles. Conditions were identical to those of Fig. 2 with 14.5 μM cytochrome c (5% reduced) plus 4 μM ubiquinone-2. The traces are successive light-dark cycles of the same sample, but with increasing concentrations of FCCP added.

of:

$$2[\text{ferrocytochrome } c^{2+}] + Q + 2H^+ \rightarrow 2[\text{ferricytochrome } c^{3+}] + QH_2 \quad (1)$$

This is seen as a small rapid net alkalinisation when the light is switched on. It is rapidly followed, however, by net acidification of the external medium as cyclic electron flow and proton pumping commence. When the light is switched off, reaction 1 is reversed causing a net further acidification which is followed by relaxation back to the initial state as protons leak back across the membranes into the inside. No net reaction will have occurred at the end of this sequence of events, and so may be repeated many times. By changing the number of electrons in the system, the size of the 'pool effect' relative to the pumping may easily be changed. Fig. 3 illustrates one method of doing this, where all of the cytochrome c is reduced by excess sodium isoascorbate before light activation. Fig. 3B also illustrates one further complication to the proton profiles which is often observed – that of a slow decrease from one steady-state level of H^+_{out} to a slightly lower one, when constant illumination is maintained. This effect was small and somewhat variable between different samples. Its cause is as yet unknown but may possibly reflect redox state-dependent binding or unbinding of cytochrome c to the surface of the vesicles in a way which causes displacement of surface protons (cf. Ref. 21).

The effects of uncoupler on proton pumping

Fig. 4 illustrates the great sensitivity of the acidification of the external medium to the uncoupler, FCCP. As the concentration is increased, the extent of acidification rapidly diminishes and the rate constant for proton leakage back across the membrane increases (as may be seen from the increased rate of decay for a given size of proton gradient when the light is switched off). Eventually, no acidification is seen at all and the only effect remaining is that of the pool effect, i.e., net alkalinisation on light activation followed by re-acidification when the light is switched off.

Effects of inhibitors

The inhibitors of the cytochrome b-c_1 complex, antimycin A, myxothiazol and DBMIB, all prevented the pumping process. As in the case of excess uncoupler, the only change in H^+_{out} in their presence was the alkalinisation caused by net ubiquinone-2 reduction. When the light was switched off, however, re-acidification was extremely slow, since the enzymic route of re-reduction of cytochrome c by quinol through the cytochrome b-c_1 complex had been blocked. Myxothiazol was more potent than antimycin A in this respect, presumably since the small antimycin A-leak [22] is absent in the myxothiazol-inhibited system.

TABLE I

APPARENT SECOND-ORDER RATE CONSTANTS

Reactions were carried out in a medium containing 160 mM sucrose, 40 mM KCl, 0.5 mM EDTA, 1 mM KCN and 0.1 μg/ml valinomycin at pH 7.2 and 23°C.

Reaction	Conditions	Value ($M^{-1} \cdot s^{-1}$) at 23°C
(1) c^{2+} → reaction centre	low [ferrocytochrome c^{2+}] high [ubiquinone-2] + antimycin A	$3.3 \cdot 10^7$
(2) Reaction centre → Q-2	high [ferrocytochrome c^{2+}] low [ubiquinone-2] + antimycin A	$\geqslant 2.4 \cdot 10^8$
(3) QH_2-2 → b-c_1	low [ubiquinol-2] high [ferricytochrome c^{3+}]	$5.7 \cdot 10^7$
(4) cytochrome b-c_1 → c	high [ubiquinol-2] low [ferricytochrome c^{3+}]	$2.2 \cdot 10^8$

Re-reduction in the presence of DBMIB when the light was switched off was relatively rapid, presumably because of its catalytic effect on the chemical non-enzymatic re-reduction rate [23].

Measurements of rate constants

It proved possible to measure the four apparent second-order rate constants of the system, as summarised in Table I. In each case, a rate of cytochrome c reduction or oxidation at 550 nm was monitored, with conditions adjusted so that the particular reaction of interest was made to be rate limiting. The pseudo-first-order kinetics of the cytochrome c were computer analysed and rate constants were extracted from semilogarithmic plots of the data and assuming that all reaction centres or cytochrome b-c_1 complexes were involved in the reactions. The latter assumption is probably incorrect, since some of the complexes are likely to be oriented with their cytochrome c sites accessible only from the vesicle interior. The apparent rate constants obtained nevertheless proved to be useful, since they allowed calculation of rate-limiting steps, steady-state fluxes and redox states of components which could be cross-checked by experimental measurement.

Sidedness of reaction sites

Cytochrome c^{3+} is added in these experiments after the vesicles have been fully formed and sealed. It is therefore reasonable to deduce that it may react only with sites exposed to the outer aqueous

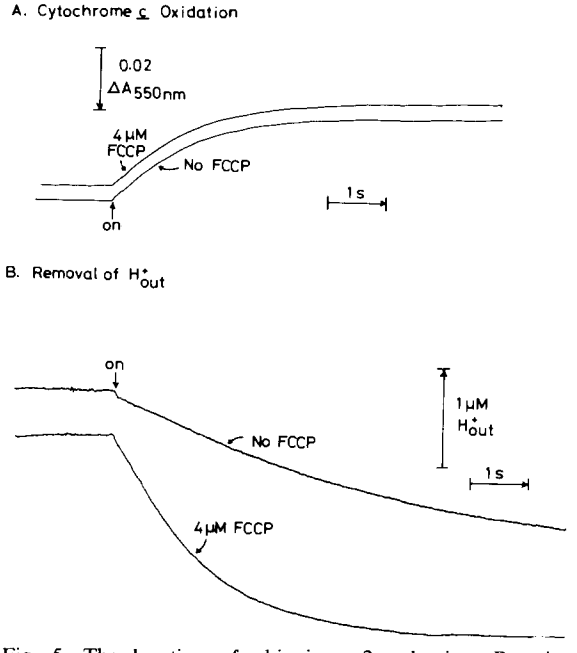

Fig. 5. The location of ubiquinone-2 reduction. Reaction medium was 160 mM sucrose, 40 mM KCl and 0.5 mM EDTA at pH 7.2 and 23°C. Other additions were 0.1 μg/ml valinomycin, 1 μg/ml antimycin A, 1 mM KCN, 16.3 μM cytochrome c (5% reduced), 4.3 μM ubiquinone-2 and vesicles to a final reaction centre concentration of 42.5 nM. In A, the rate of oxidation of cytochrome c was monitored at 550 nm when the light was switched on. In B, 100 μM phenol red was added and the rate of proton removal from the outside medium was monitored at 542 nm. Initial rates of cytochrome c oxidation were 984 $nM \cdot s^{-1}$ in the presence or absence of 4 μM FCCP. Initial rate of proton removal was 240 $nM \cdot s^{-1}$ in the absence of FCCP which increased to 990 $nM \cdot s^{-1}$ when 4 μM FCCP was present.

buffer. The ubiquinone-2, however, is easily able to cross the membrane [24] and hence may react with reaction sites on either side of the vesicles. Experiments were performed to ascertain the sidedness of the ubiquinone-2 reactions.

The location of ubiquinone-2 reduction. The experiment illustrated in Fig. 5 demonstrates that the ubiquinone-2 is predominantly reduced at a site in contact with the internal aqueous phase of the vesicles. The experiments were performed in the presence of antimycin A so that illumination caused net reduction of ubiquinone-2 with only a slow back-reaction. When such a reaction was monitored by direct measurement of ferrocytochrome c^{2+} oxidation (Fig. 5A), no effect of uncoupler was observed. When, however, the reaction was monitored by the proton removal, which should occur concomitantly with the ubiquinone-2 reduction, then a severe underestimate of the rate was obtained and the profile had a clear lag phase (Fig. 5B). Addition of sufficient uncoupler, however, removed this lag and indicated a rate in agreement with the more direct method of cytochrome *c* measurement. It is concluded that the ubiquinone-2 is reduced at a site in contact with the internal aqueous phase of the inside of the vesicles. The lag is observed since a finite ΔpH must be set up before protons will leak inside from the external buffer. The ratio of initial rates in Fig. 5A indicates that at least 80% of quinone reduction occurs at a site in contact with the internal aqueous phase.

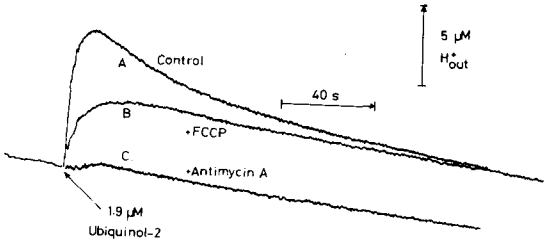

Fig. 6. The location of ubiquinol-2 oxidation. Medium used was 160 mM sucrose, 40 mM KCl and 0.5 mM EDTA at pH 7.2 and 23°C. Other additions were 0.1 μg/ml valinomycin, 1 mM KCN, 100 μM phenol red, 320 μM cytochrome *c* and vesicles to a final cytochrome *b*-c_1 concentration of 79 nM. In trace B, 4 μM FCCP was added and in trace C, 1 μg/ml antimycin A. Reaction was started by addition of a pulse of quinol and proton appearance was monitored at 542 nm. Calibration was achieved with pulses of standardised HCl.

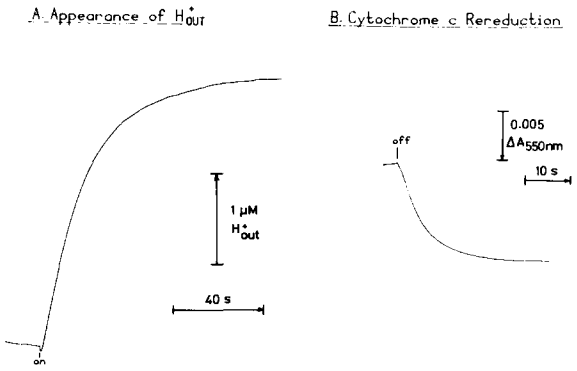

Fig. 7. Measurements of $H^+/2e^-$ ratios. The reaction medium was 160 mM sucrose, 40 mM KCl and 0.5 mM EDTA at pH 7.2 and 23°C. Other additions were 1 mM KCN, 0.1 μg/ml valinomycin, 8.2 μM cytochrome *c* (containing 530 nM ferrocytochrome c^{2+}), 2.2 μM ubiquinone-2 and vesicles to a final cytochrome *b*-c_1 concentration of 53 nM. In the top traces, 100 μM phenol red was also present so that external protons could be monitored at 542 nm. Initial rates were calculated by computer analysis of the decay curves and their extents, as described in the text. The rate of appearance of H^+_{out} was calculated to be 204 $nM \cdot s^{-1}$ and the rate of cytochrome *c* rereduction was 92 $nM \cdot s^{-1}$, giving an $H^+/2e^-$ ratio of 4.4. Proton measurements were calibrated by measuring the change produced on illumination in the presence of antimycin A. This was equated with the measured extent of cytochrome *c* oxidation which occurred on illumination under the same conditions.

The location of ubiquinol-2 oxidation. This was determined using the quinol pulse method originally used for reconstituted vesicles containing cytochrome *b*-c_1 complex [1,2]. In the presence of excess ferricytochrome c^{3+} a pulse of quinol caused a rapid proton ejection with no lag phase and an overshoot of the number of protons released (Fig. 6A). Uncoupler, however, decreased the initial rate of proton ejection by a factor of two and also removed the overshoot (Fig. 6B). This is entirely consistent with scalar and pumped protons being produced on the outside of the vesicle when quinol is added. In the presence of uncoupler, only the scalar reaction is observed (cf. Refs. 1 and 2). The site of quinol re-oxidation is therefore in contact with the external aqueous phase.

Proton / electron ratios

Pumping on addition of quinol. The data of Fig. 7 may also be used to deduce from the extent of H^+ appearance that for each pair of electrons donated to the cytochrome *b*-c_1 complex by quinol, two scalar protons are produced and 2 ± 0.2 extra

protons are 'pumped' from inside to outside, as measured from the extent of H^+ overshoot in the absence of uncoupler (cf Refs. 1 and 2), i.e.:

$$QH_2 + 2[\text{ferricytochrome } c^{3+}] + 2H^+_{in} \xrightarrow{bc_1} Q + 4H^+_{out} + 2[\text{ferrocytochrome } c^{2+}] \quad (2)$$

This was checked further by running two experiments in which the initial rate of cytochrome *c* reduction (at 550 nm) and the initial rate of proton ejection (at 542 nm with 100 μM phenol red added) were measured, on addition of a pulse of quinol. The result gave a value of 4.4 $H^+/2e^-$, in agreement with the extent measurements above.

Light-activated proton pumping. Estimates were also made of light-activated proton pumping and electron-transfer rates so that the $H^+/2e^-$ ratios could be calculated. The initial $H^+/2e^-$ ratio was calculated at that point soon after switching the light on when the pumping had just counteracted the opposing 'pool effect' so that the H^+_{out} concentration had transiently returned to its initial value. To obtain the rate of pumping at this point, the data were computer plotted as a semilogarithmic plot. Such a plot was linear after the short initial lag caused by the pool effect and hence a rate constant could be obtained. The rate of pumping could then be found by extrapolation of the linear region to the time point at which the rate was required. The rate of electron cycling at this same time point was obtained in a separate experiment in the absence of phenol red so that cytochrome *c* redox changes could be monitored. The sample was illuminated for the appropriate length of time and the light was then switched off so that the cytochrome *c* became re-reduced (Fig. 7B). The semilogarithmic plot of this decay was linear after a small lag, caused by the presence of some $P\text{-}870^+$ in the illuminated state which re-oxidised the cytochrome *c* for a short time after the light was switched off. By extrapolating the linear region of the semilogarithmic plot to the point at which the light was switched off, the initial rate of cytochrome *c* re-reduction, and hence the rate of electron cycling, could be ascertained.

Typical results of such calculations are given in the legend to Fig. 7, and gave an $H^+/2e^-$ ratio close to four. This is expected from a reaction in which two protons are moved because of the vectorial nature of reaction sites with an additional two protons translocated by the proton-motive ability of the cytochrome b-c_1 complex.

Discussion

The present experimental system has been developed from two types of work already detailed in the literature. The first of these has been the successful re-incorporation of the cytochrome b-c_1 complex into lipid vesicles in such a way that its energy-transducing function is restored [1–3,19,25]. The second type of development has been the production in solution of a cyclic electron-transport system involving the cytochrome b-c_1 complex and bacterial reaction centres [11,12]. The resulting combination of these techniques has now produced a well defined system in which the energy-transducing events of the cytochrome b-c_1 complex systems may be studied.

The results reported here provide an initial kinetic characterisation. The rate constants are such that, in general, cyclic electron flow is limited by the reduction of cytochrome b-c_1 complex by ubiquinol-2. The system can be changed, however, so that cytochrome *c* reduction by the cytochrome b-c_1 complex becomes rate limiting. In practice, the rate-limiting step may be changed by altering relative amounts of components, light intensity and number of electrons (for example, by redox poising) contained in the system. In this way, a very versatile experimental system is produced.

On initiation of electron flow around the system, four protons appear in the external medium for each pair of electrons which pass through the cytochrome b-c_1 complex. Experimentally, values slightly in excess of 4 were obtained consistently. This overestimate probably arises from the calibration method for proton measurements. This calibration always used total buffering power of outer phase plus trapped phase. In most of the experiments in the coupled state, however, the trapped phase would contribute little to the buffering seen by the protons which appear rapidly on the outside. Hence, H^+_{out} would be consistently overestimated giving an overestimate also of $H^+/2e^-$. This error was estimated to be between 5 and 10%, as judged by the extent of acidification

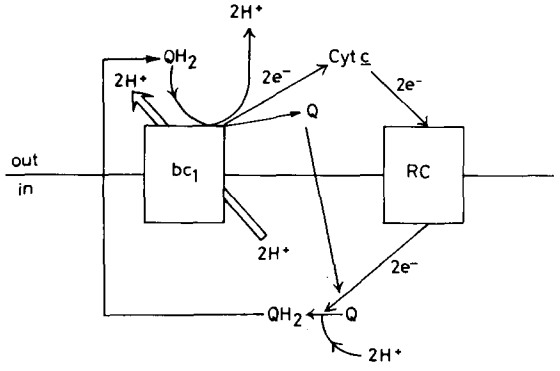

Fig. 8. Schematic representation of the mechanism of operation of the vesicle system. Sides of the vesicle with which reactions are in contact are indicated, with ubiquinone and ubiquinol being freely diffusible across the membrane. Net proton movement will be $4H^+/2e^-$, with 2 H^+ moved by vectorial arrangement of carriers and a further 2 H^+ moved by a proton-motive function of the cytochrome b-c_1 complex. RC, reaction centre.

overshoot and subsequent relaxation caused by pulses of HCl. Two of the four protons observed in the coupled state are to be expected from the demonstrated vectorial arrangement of quinone reduction and oxidation sites (see Fig. 8). The second two protons appear because of the additional proton-motive ability of the cytochrome b-c_1 complex which has already been demonstrated to move an extra $2H^+/2e^-$ [7,26,27].

It may be noted from Fig. 2 that significant cyclic electron transfer is possible with cytochrome c alone or with ubiquinone-2 alone, even although both are required for optimal activity. The result with cytochrome c alone may be explained by there being sufficient residual ubiquinone-10 in both the cytochrome b-c_1 complex and reaction centre preparations to allow direct electron transfer between them. Direct transfer in the soluble reconstituted system from reaction centre to cytochrome b-c_1 complex has been demonstrated already by Packham et al. [11]. The result with ubiquinone alone is rather more difficult to explain, but one possibility is that ubiquinone-2 is able to act as a mediator between cytochrome b-c_1 and reaction centre in the absence of added cytochrome c.

Acknowledgements

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References

1 Leung, K.H. and Hinkle, P.C. (1975) J. Biol. Chem. 250, 8467–8471
2 Guerrieri, F. and Nelson, B.D. (1975) FEBS Lett. 54, 339–342
3 Wielburski, A. and Nelson, B.D. (1978) FEBS Lett. 93, 69–72
4 Hurt, E.C., Hauska, G. and Shahak, Y. (1982) FEBS Lett. 149, 211–216
5 Serrano, R., Kanner, B.I. and Racker, E. (1976) J. Biol. Chem. 248, 5841–5847
6 Carroll, R.C. and Racker, E. (1977) J. Biol. Chem. 252, 6981–6990
7 Wikström, M.K.F., Krab, K. and Saraste, M. (1981) Annu. Rev. Biochem. 50, 623–655
8 Racker, E. and Stoekenius, W. (1974) J. Biol. Chem. 249, 662–663
9 Schonfeld, M., Montal, M. and Feher, G. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 6351–6355
10 Packham, N.K., Packham, C., Mueller, P., Tiede, D.M. and Dutton, P.L. (1980) FEBS Lett. 110, 101–106
11 Packham, N.K., Tiede, D.M., Mueller, P. and Dutton, P.L. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 6339–6343
12 Matsuura, K., Packham, N.K., Mueller, P. and Dutton, P.L. (1981) FEBS Lett. 131, 17–22
13 Hatefi, Y. and Rieske, J.S. (1967) Methods Enzymol. 10, 225–229
14 Hatefi, Y. and Rieske, J.S. (1967) Methods Enzymol. 10, 242–245
15 Clayton, R.K. and Wang, R.T. (1971) Methods Enzymol. 23, 696–704
16 Rich, P.R. (1981) Biochim. Biophys. Acta 637, 28–33
17 Kagawa, Y. and Racker, E. (1971) J. Biol. Chem. 246, 5477–5487
18 Bangham, A.D. (1982) Tech. Lipid Membrane Biochem., Tech. Life Sci. B420, 1–25
19 Rich, P.R. and Clarke, S.D. (1982) FEBS Lett. 148, 54–58
20 Jackson, J.B., Crofts, A.R. and Von Stedingk, L.-V. (1968) Eur. J. Biochem. 6, 41–54
21 Mitchell, P. and Moyle, J. (1983) FEBS Lett. 151, 167–178
22 Chance, B., Boveris, A., Oshino, N. and Loschen, G. (1973) in Oxidases and Related Redox Systems (King, T.E., Mason, H.S. and Morrison, M., eds.), pp. 350–353, University Press, Baltimore

23 Rich, P.R. and Bendall, D.S. (1980) Biochim. Biophys. Acta 592, 506–518
24 Futami, A., Hurt, E. and Hauska, G. (1979) Biochim. Biophys. Acta 547, 583–596
25 Von Jagow, G., Engel, W.D., Schägger, H. and Becker, W.F. (1982) in Function of Quinones in Energy Conserving Systems (Trumpower, B.L., ed.), pp. 351–364, Academic Press, New York
26 Brand, M.D. (1977) Biochem. Soc. Trans. 5, 1615–1620
27 Mitchell, P. (1976) J. Theor. Biol. 62, 327–367