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IDENTIFICATION OF UBIQUINONE AS THE SECONDARY ELECTRON ACCEPTOR IN THE PHOTOSYNTHETIC APPARATUS OF *CHROMATIUM VINOSUM*

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SUMMARY

Extracting *Chromatium vinosum* chromatophores with light petroleum destroys their ability to perform photochemistry on the second of two closely-spaced actinic flashes, without affecting photochemistry on the first flash. Extraction also increases the likelihood of a back-reaction in which an electron returns from the primary electron acceptor directly to P_{870}. These effects probably reflect the removal of a secondary electron acceptor. Extraction does not appear to interfere with the primary photochemical reaction. Reconstituting the extracted chromatophores with the lipid extract or with pure ubiquinone (Q) completely reverses the effects of the extraction. Chromatography of the lipid extract shows that Q is the only active material that it contains in detectable quantity. These observations support the conclusion that Q is the secondary electron acceptor.

Piericidin A, certain alkyl-substituted quinolinequinones, and a substituted 4,7-dioxobenzothiazole inhibit electron transfer between the primary and secondary acceptors. The sensitivity to these inhibitors, and the participation of Q and non-heme iron suggest that the secondary electron-transfer reaction resembles the reactions catalyzed by respiratory dehydrogenases.

The proton uptake that follows flash excitation does not seem to be tightly linked to the reduction of the secondary electron acceptor. It still occurs (though with decreased amplitude) in extracted chromatophores, and even in the presence of inhibitors of the secondary electron-transfer reaction.

INTRODUCTION

Following the discovery [1] that photosynthetic bacteria contain large amounts of ubiquinone (Q), and the observation [2, 3] that illumination of chromatophores causes ultraviolet absorbance changes suggestive of the reduction of Q, the role of Q

Abbreviations: E_m and E_{m7}, midpoint redox potential, with 7 specifying the pH; PMS, *N*-methylphenazonium methosulfate; Q, Q-6, and Q-7, ubiquinone, with 6 or 7 specifying the number of isoprenoid units in the side chain.

in bacterial photosynthesis has provoked considerable discussion. One recurring suggestion has been that Q acts as the electron acceptor in the primary electron-transfer reaction, the photooxidation of P_{870} [2, 4–7]. The extraction of Q from chromatophores destroys their ability to carry out photophosphorylation, and alters the light-induced ultraviolet absorbance changes [8–11]. Even the removal of most or all of Q, however, does not prevent chromatophores or subchromatophore particles from performing the photooxidation of P_{870} [10–13].

The removal of Q from subchromatophore particles does damage secondary electron transfer reactions, and the addition of Q to the extracted preparations reverses this effect [11, 13–15]. The present report describes extraction and reconstitution experiments with chromatophores of *Chromatium vinosum*. The results support the identification of Q as the secondary electron acceptor which previous reports from this laboratory [16–20] have called "Y".

MATERIALS AND METHODS

C. vinosum was grown photoautotrophically as described previously [19]. Chromatophores were prepared from 3-day cultures by differential centrifugation following sonication of cell suspensions, either in 0.4 M sucrose containing 0.1 M Tris–HCl, pH 7.5, or in 0.05 M Tris–HCl, pH 8.2. The chromatophore preparations were freed of salts by passage through a column of Sephadex G-25 in water, lyophilized, and stored in a vacuum desiccator at 4 °C in the dark.

Ubiquinone was extracted typically by adding 35 ml of light petroleum to 50 mg of lyophilized chromatophores (containing approx. 0.7 mg bacteriochlorophyll), letting the mixture stand for 30 min at room temperature, and then centrifuging. The pellet was washed twice by shaking with 35 ml portions of light petroleum and centrifuging, and then dried in vacuo. For measurements of photochemical activity, 5-mg samples of the extracted material were resuspended in 3 ml of water or 0.05 M Tris–HCl, pH 8.1. The suspensions were often somewhat turbid; they could be clarified by very brief sonication.

Light petroleum alone sometimes was ineffective in extracting Q from chromatophores that had been prepared with sucrose. The inclusion of a small amount of methanol in the light petroleum led to an effective extraction, with optimal proportions of the two solvents varying from preparation to preparation, probably as a function of the amounts of sucrose and buffer that remained with the chromatophores. Excess methanol in the mixture caused extraction of the bacteriochlorophyll and denaturation of the chromatophores. The figure legends and the text provide additional details on these variations of the extraction procedure.

For reconstitution, 0.3 ml of light petroleum typically containing 0.15 μmole of ubiquinone with six isoprenoid units in the side chain (Q-6) was added to 5 mg of extracted chromatophores in a 5-ml beaker. The light petroleum was allowed to evaporate in a desiccator, and then drying was continued at reduced pressure. The reconstituted chromatophores were taken up in water or Tris–HCl buffer.

Chromatography of the lipid extracts on silica gel thin-layer plates, Decalso columns, and paper followed standard published procedures [21–24]. Bacteriochlorophyll was determined spectrophotometrically at 767 nm, after extraction into acetone–methanol (7 : 2, v/v) [25]. Q was determined by the change in absorbance at 275 nm

on addition of $NaBH_4$ to a solution in light petroleum–95 % ethanol (1:2, v/v) [26]. Measurements of the photooxidation of P_{870} and cytochrome C_{555}, and measurements and control of the redox potential used techniques which have been described previously [16, 19]. The measurements of proton uptake after flash excitation employed phenol red as a pH indicator, as described by Cogdell et al. [27]. The Dam–Karrer reaction was performed as described by Irrevere and Sullivan [28], except that the alkaline ethanol solution was prepared with NaOH instead of sodium.

Piericidin A and the synthetic quinones that were used for the experiments of Figs 2 and 5 were generous gifts from Drs Karl Folkers and Thomas Porter, of the University of Texas at Austin. Syntheses of these compounds are described in refs 29–32. Phylloquinone, Q-6 and menadione were Sigma preparations.

RESULTS

Fig. 1A shows measurements of oxidation of cytochrome C_{555} on each of two saturating laser flashes, 15 ms apart. Photochemical activity on the second flash is a measure of the ability of a secondary acceptor (Y) to remove an electron from the primary acceptor (X) in the interval between the two flashes [16]. In agreement with previous reports [15, 17], this reaction is blocked almost completely by the addition

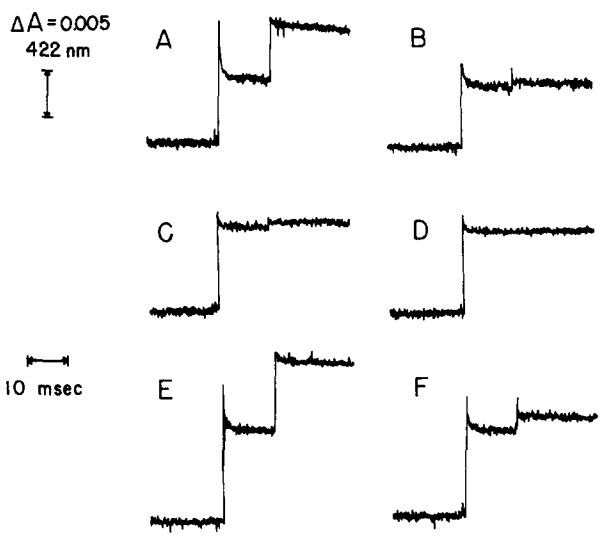

Fig. 1. Cytochrome oxidation occurring on exposure of *C. vinosum* chromatophores to two flashes, 15 ms apart. Each sample contained chromatophores (as described below) in 4.2 ml 0.1 M KCl with 48 μM phenol red and 12 μM PMS. The suspensions were clear without sonication and had a pH of 7.0±0.1 in all cases. They were made anaerobic by bubbling with N_2. Each trace shows an average of five measurements, prepared by a computer of average transients. An upward deflection indicates an absorbance decrease at 422 nm. Baseline drift in the absence of the flashes has been subtracted in the computer. (A), 4.8 mg lyophilized chromatophores (prepared with sucrose; not extracted); (B), same sample as in A, after addition of 360 μM *o*-phenanthroline; (C), 5.0 mg extracted chromatophores (the chromatophore preparation used for A was extracted with 0.7 % methanol in light petroleum, as described under Materials and Methods); (D), same as C, after addition of 360 μM *o*-phenanthroline; (E), 5.0 mg extracted chromatophores (as in B) reconstituted with 0.15 μmole Q-6; (F), same as E, after addition of 360 μM *o*-phenanthroline.

of 360 μM *o*-phenanthroline (Fig. 1B). Extraction of the chromatophores with 0.7 % methanol in light petroleum has the same effect (Fig. 1C). Chromatophores that have been both extracted and treated with *o*-phenanthroline show no detectable activity on the second flash (Fig. 1D).

The recombination of the light petroleum extracts with the extracted chromatophores restores cytochrome photooxidation on the second flash. The lipid extract can be replaced by Q-6 (Fig. 1E), or less effectively by phylloquinone (vitamin K_1) or menadione (see below). In all cases, the reconstituted activity retains sensitivity to *o*-phenanthroline (Fig. 1F).

None of these treatments significantly affects the extent of cytochrome photooxidation on the first flash. (Fig. 1 does not demonstrate this clearly because part of the cytochrome that is oxidized on the first flash becomes reduced again rapidly. Some of the traces do not distinguish absorbance changes due to this oxidation and reduction from flash artifacts.)

The effects of extraction cannot simply reflect a role of Q in buffering the redox potential of the chromatophores (cf. ref. 33). Suspensions of chromatophores under N_2 and in the presence of 10 μM *N*-methylphenazonium methosulfate (PMS) established a measured redox potential of approx. +200 mV, well below the midpoint redox potential (E_m) (+341 mV, ref. 19) of cytochrome C_{555}. Untreated, extracted. and reconstituted chromatophores all behaved alike in this respect.

Fig. 2 shows a different measure of the activity of the secondary electron acceptor. For this experiment, the redox potential was raised to +430 mV, so as to oxidize cytochrome C_{555} chemically. Under these conditions, a single flash causes P_{870} photooxidation, but the return of P_{870}^+ to the reduced state is very slow (Fig. 2A). The cytochrome is unable to provide an electron, and electron flow from Y to P_{870}^+ apparently cannot occur rapidly. If one prevents forward flow of electrons from X^- to Y, however, a rapid back-reaction occurs in which an electron returns

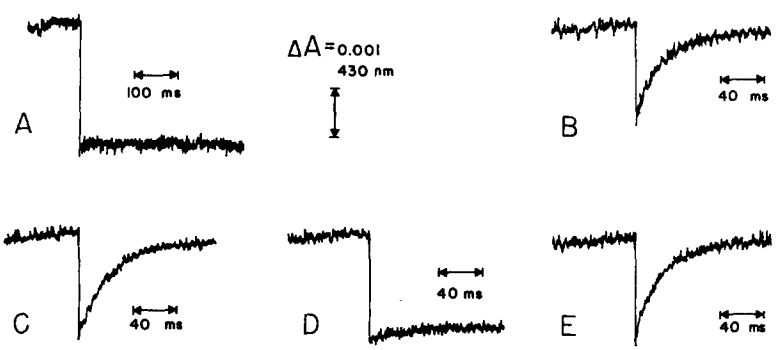

Fig. 2. Kinetics of reduction of P_{870}^+ after single flashes at high redox potentials. Each sample contained chromatophores (as described below) in 8.2 ml of anaerobic 0.1 M KCl with 12 μM PMS, $K_4Fe(CN)_6$ (initially 12 μM), and sufficient $K_3Fe(CN)_6$ (approx. 24 μM) to raise the redox potential to 430$\pm$10 mV. Each trace is an average of five measurements, with a downward deflection indicating an absorbance increase at 430 nm. Such an absorbance change reflects the oxidation of P_{870}. A, 10.6 mg lyophilized chromatophores (as in Fig. 1A); B, same as A, after addition of 54 μM 7-*n*-pentadecyl-6-hydroxy-5,8-quinolinequinone; C, 10.3 mg extracted chromatophores (as in Fig. 1C); D, 10.5 mg extracted chromatophores, reconstituted with 0.3 μmole Q-6; E, same as D, after addition of 54 μM 7-*n*-pentadecyl-6-hydroxy-5,8-quinolinequinone.

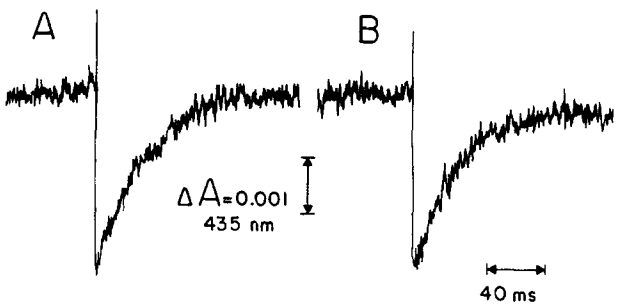

Fig. 3. P_{870} photooxidation at low temperature. Each sample contained 3.4 mg (dry wt) chromatophores per ml of (glycerol: 0.1 M Tris–HCl, pH 7.7) (1:1, v/v), in a 1-mm thick cuvette, held in a Dewar at 45° with respect to the excitation and measuring light beams (which were orthogonal to each other). Fins of the cuvette extended downward into a bath of liquid N_2. A thermocouple in the cuvette provided a measure of the temperature (100±10 °K for both traces). Each trace shows an average of 20 measurements with saturating laser flashes. The samples were kept in darkness for several minutes at room temperature before they were cooled. A, lyophilized chromatophores, prepared without sucrose; B, same preparation of chromatophores, extracted exhaustively with light petroleum at room temperature. The extracted chromatophores exhibited only negligible cytochrome photooxidation on the second of two flashes, approximately 2 % of that on the first flash, 4 ms earlier (for the cytochrome measurement, the chromatophores were suspended in anaerobic 33 mM KCl, 67 mM Tris–HCl, pH 7.7, with 3 μM PMS).

directly from X^- to P_{870}^+ [11, 13, 15]. This can be achieved by adding *o*-phenanthroline or, as Fig. 2B shows, 7-*n*-pentadecyl-6-hydroxy-5,8-quinolinequinone (see also Fig. 6). Extracting the chromatophores has the same effect (Fig. 2C). Reconstituting the extracted chromatophores with Q-6 restores the original, slow kinetics (Fig. 2D), and the reconstituted chromatophores retain sensitivity to the same inhibitor (Fig. 2E).

Another way to block the electron transfer between X^- and Y is to lower the temperature. Liquid N_2 temperatures prevent the photooxidation of cytochrome C_{555} as well, allowing one to measure the back-reaction between X^- and P_{870}^+ at more moderate redox potentials. Fig. 3 illustrates the photooxidation of P_{870} and the back-reaction, following single flashes under these conditions. Both the extent of the initial photooxidation and the kinetics of the back-reaction are the same in extracted chromatophores as they are in untreated chromatophores.

Fig. 4A shows the relationship between the amount of Q that the extraction removes from the chromatophores and the disappearance of Y activity. At least 75 % of total Q can be removed without affecting the ability of the chromatophores to oxidize cytochrome on a second flash. To reduce the photochemical activity by 50 %, one must extract 90 % of the Q. One can rationalize these results in terms of the wealth of Q that occurs in *Chromatium* chromatophores. Fuller et al. [21] found a ratio of Q to bacteriochlorophyll of 0.21, and our measurements gave a ratio of 0.16, in rough agreement with theirs. This would imply a 5–10-fold excess of Q over P_{870}, assuming a photosynthetic unit of 100–200 bacteriochlorophyll molecules. Ke and Chaney [34] reported a 5-fold excess of Q over P_{870} in a photochemically active subchromatophore fraction from *C. vinosum*. Cusanovich and Kamen [35] found somewhat lower concentrations of Q in chromatophores, but still obtained a substantial excess over P_{870}. A small fraction of the Q pool might suffice to insure

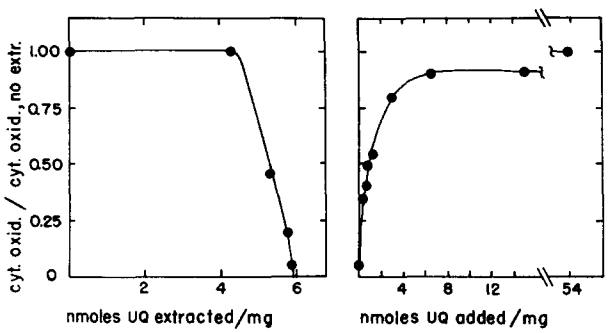

Fig. 4. (A) Loss of secondary acceptor activity as a function of the amount of Q extracted by light petroleum. A 100-mg sample of lyophilized chromatophores (prepared without sucrose) was extracted with successive 10-ml portions of light petroleum by grinding in a mortar. The first three extractions were performed at 0 °C; the fourth at 22 °C. The solvent was removed by filtration after each extraction. (Separate experiments showed that extraction with light petroleum at room temperature removed virtually all of the Q from chromatophores that had been prepared as these were. Further extraction with hot methanol followed by a mixture of hot methanol and ether released no more. Extraction with boiling light petroleum in a Soxhlet apparatus released the same amount of Q as did the extraction at room temperature). The ordinate gives the ratio of the amount of cytochrome oxidation that occurred on a second actinic flash to that which occurred in the unextracted chromatophores. Cytochrome oxidation was measured from absorbance changes at 422 nm, as in Fig. 1, with a 4-ms interval between the two flashes. Each sample contained 30–40 μM bacteriochlorophyll in 0.05 M Tris–HCl, pH 8.1, with 5 μM PMS. The data are corrected for slight differences in the bacteriochlorophyll concentration. The abscissa scale refers to the dry weight of the chromatophores. (B) Recovery of secondary acceptor activity, as a function of the amount of Q-6 added to extracted chromatophores. The fully extracted sample that was obtained in part A of this experiment was divided into portions of 5 mg each and reconstituted with various amounts of Q-6. The ordinate and abscissa scales and the conditions of the measurements are the same as in A.

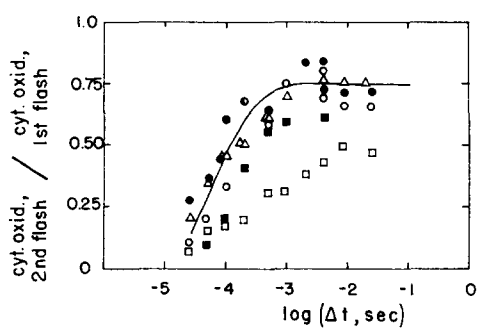

Fig. 5. Kinetics of the secondary electron transfer reaction in reconstituted chromatophores. The ordinate gives the ratio of the amount of cytochrome photooxidation observed on a second actinic flash to that observed on the first flash. The abscissa gives, on a logarithmic scale, the interval (in s) between the two flashes. Conditions were as in Fig. 4. ○, lyophilized chromatophores (prepared without sucrose and not extracted; ●, light petroleum-extracted chromatophores, reconstituted with 0.11 μmole Q-6 mg dry weight; ■, light petroleum-extracted chromatophores, reconstituted with the material extracted by light petroleum (equivalent to 0.5 μmole Q/mg chromatophores); △, light petroleum-extracted chromatophores reconstituted with 2.7 μmoles phylloquinone/mg; □, light petroleum-extracted chromatophores, reconstituted with 1.5 μmoles menadione/mg.

rapid removal of electrons from the primary acceptor.

Reconstitution of 50 % of the original Y activity occurs upon the addition of Q-6 in an amount equal to about 16 % of the original content of Q (Fig. 4B). Restoration of 90 % of the activity requires the addition of an amount approximately equal to the original content. On a molar basis, the exogenous Q therefore is somewhat less effective in restoring activity than the endogenous Q is in maintaining the original activity (cf. Fig. 4A). This is not surprising, because the reconstitution procedure probably succeeds in returning to the chromatophores only a small part of the Q that one adds. The restoration of NADH oxidase activity in extracted submitochondrial particles requires the addition of a much greater excess of Q [36].

As Fig. 5 shows, phylloquinone was essentially as effective as Q-6 in reconstituting chromatophores that had been extracted with light petroleum under mild conditions. In both cases, the maximum amount of cytochrome oxidation that occurred on a second flash was 75–80 % of that which occurred on the first. The same range is typical of unextracted chromatophores (Figs 5 and 6, and refs 16 and 19). Chromatophores that had been extracted more vigorously (for 4 h with refluxing light petroleum in a Soxhlet apparatus) responded less well to phylloquinone. When these chromatophores were reconstituted with Q, the amount of cytochrome oxidation that occurred on the second flash returned to 77 % of that which occurred on the first. When they were reconstituted with saturating amounts of phylloquinone (0.1–

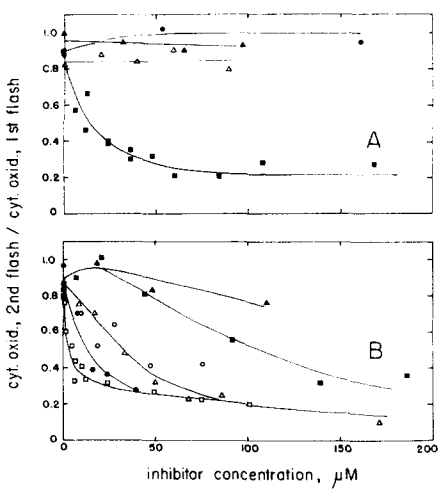

Fig. 6. Inhibition of the secondary electron-transfer reaction by piericidin A and substituted -iuq nones. Cytochrome oxidation on each of two flashes 1 ms apart was measured in chromatophores that had not been lyophilized or extracted. Each sample contained 50 μM bactericchlorophylli in 3.05 ml of anaerobic 0.4 M sucrose with 0.1 M Tris–HCl, pH 7.7, and 5 μM PMS. The ordinate gives the ratio of the cytochrome oxidized on the second flash to that oxidized on the first.The inhibitores were added in small portions of ethanol. (A) ●, 6-phytyl-5-hydroxy-2,3-dimethyl-1,4- benzoquinone; ▲, 6-ω-cyclohexylpentyl-5-hydroxy-2,3-dimethoxy-1,4-benzoquinone; △, 5-*n*-dodecylmercapto-2,3-dimethoxy-1,4-benzoquinone; ■, piericidin A. (B) ■, 7-*n*-tetradecylmercapto-6-hydroxy-5,8-quinolinequinone; ▲, 6-ω-cyclohexyloctyl-7-hydroxy-5,8-quinolinequinone; △, 7-*n*-pentadecyl-6-hydroxy-5,8-quinolinequinone; ○, 6-cycloheptylamino-2-methyl-5,8-quinolinequinone; ●, 6-*n*-dodecylamino-5,8-quinolinequinone; □, 5-*n*-pentadecyl-6-hydroxy-4,7-dioxobenzothiazole.

1 μmole/mg), the second flash gave only 49 % as much cytochrome oxidation as the first. Menadione was measurably less effective than Q, even in chromatophores that had been extracted gently (Fig. 5).

One can study the kinetics of electron transfer between the primary and secondary electron carriers by measuring the amount of cytochrome oxidation that occurs on the second flash, as a function of the time that elapses between the two flashes [16]. Fig. 5 shows that the kinetics are the same in chromatophores that have been extracted and reconstituted with Q or phylloquinone as they are in unextracted chromatophores. Reconstitution with menadione results in slower kinetics. These results distinguish Q and phylloquinone from PMS and methylene blue, both of which can serve as secondary electron acceptors in subchromatophore preparations from *C. vinosum* [18]. The kinetics of the reaction with PMS and methylene blue are considerably slower. In addition, electron transfer to PMS and methylene blue is insensitive to *o*-phenanthroline [18], whereas 1–2 mM *o*-phenanthroline abolishes electron transfer to any of the quinones.

Because phylloquinone was able to reconstitute extracted chromatophores, and because *Chromatium* has been reported [1, 21, 37, 38] to contain menaquinone (vitamin K_2), we examined the quinone content of the lipid extracts. Lyophilized chromatophores (prepared with sucrose) were extracted exhaustively with 0.5 or 0.7 % methanol in light petroleum, and the extracts were taken to dryness under Ar and chromatographed on silica gel thin-layer plates with chloroform–benzene (1:1, v/v) as the solvent. Samples of Q-6 and phylloquinone were chromatographed on the same plates as standards. The silica gel was scraped off the plates in five fractions, and the adsorbed materials were eluted with ether. One of the fractions contained Q, which has been reported to be ubiquinone with seven isoprenoid units in the side chain (Q-7) in *C. vinosum* [1, 21, 38]. The Q was visibly yellow on the plate, with R_F 0.3–0.4, and the absorption spectra of the eluate before and after the addition of $NaBH_4$ were essentially identical with those of pure Q. A second fraction included the section of the plate with R_F values of 0.50–0.65. This area would contain menaquinone, if it were present in the extract (phylloquinone had R_F 0.57); but nothing was visible in this area of the plate, either under ordinary light or under an ultraviolet lamp. The eluate had very little ultraviolet absorption. The three remaining fractions contained unidentified colored components with R_F values of 0.1, 0.44, and 0.73. Together the five fractions accounted for essentially the entire plate, excluding only thin regions that were left between them to minimize cross-contamination.

The eluates from each of the fractions were dried, redissolved in light petroleum, and tested for their ability to reconstitute extracted chromatophores, as judged from cytochrome photooxidation in double-flash experiments. Only one of the five samples gave reconstitution. This was the one that contained Q. We conclude that the chromatophores do not contain detectable amounts of menaquinone, and that Q accounts for the activity of the extracts. Similar results were obtained when chromatophores (or crude cell sonicates from which sulfur granules had been removed by centrifugation) were extracted with different techniques (with light petroleum in a Soxhlet apparatus or with boiling methanol) and when the extracts were chromatographed on Decalso columns or on paper. We did not find any material with the spectral characteristics of menaquinone or phylloquinone, nor did we find any material which gave their characteristic color in the Dam–Karrer reaction.

The conclusion that *C. vinosum* lacks menaquinone disagrees with the conclusions of previous investigators [1, 21, 37, 38]. Some of the earlier studies were not rigorous in their purification and identification of menaquinone, but others appear to have been exemplary. We can only suggest that the quinone content of *C. vinosum* may vary with culture conditions or the age of the culture, as do the rhodoquinone and ubiquinone content of *Rhodospirillum rubrum* [39–41]. Earlier investigators generally used a somewhat more complex growth medium than we do, and some studied cultures that probably were in a very late stationary phase.

Current evidence suggests that the primary electron acceptor in *C. vinosum* may be a non-heme iron complex [42]. The involvement of both non-heme iron and Q suggested similarities between the photosynthetic system and the dehydrogenases of the mitochondrial respiratory chain. A sensitivity to *o*-phenanthroline is also a property of mitochondrial NADH dehydrogenase [43]. Pursuing this theme, we found that piericidin A (a substituted 4-hydroxypyridine which blocks electron flow between non-heme iron and Q in NADH dehydrogenase [44, 45]) inhibited the secondary electron transfer in chromatophores (Fig. 6A). Certain substituted 5,8-quinolinequinones also proved to be effective inhibitors (Figs 2 and 6B). These compounds inhibit NADH oxidase and succinate oxidase in mitochondria, and some are effective as antibiotics against the malarial microorganisms *Plasmodium berghei* and *Plasmodium gallinaceum*, which contain Q but lack naphthoquinones [29, 30]. 5-*n*-Pentadecyl-6-hydroxy-4,7-dioxobenzothiazole, the most potent of the inhibitors shown in Fig. 6, has also been found to be an effective inhibitor of two Q-enzyme systems in mitochondria (Folkers, K., personal communication). Benzoquinones related to the quinolinequinones were not inhibitory (Fig. 6A). 7-*n*-Heptyl-8-hydroxyquinoline-*N*-oxide was not inhibitory. Rotenone, an inhibitor of NADH dehydrogenase which is structurally unrelated to piericidin, also did not inhibit the chromatophore reaction.

The dependence of the apparent E_m values of X and Y on pH and ionic strength suggests that the uptake of a proton accompanies the reduction of either of these carriers [19, 20, 46]. Proton uptake does occur following flash excitation of chromatophores [27, 47, 48], and an understanding of this phenomenon may be critical for an understanding of the mechanism of photophosphorylation. The proton uptake has appeared to be linked somehow to the reduction of Y, because it is inhibited by *o*-phenanthroline and it is diminished in subchromatophore preparations that are deficient in the secondary electron acceptor [50, 51]. We therefore investigated the effects on proton uptake of removing Y by extraction.

Fig. 7 shows measurements of the proton uptake that followed each of two laser flashes, with untreated, extracted, and reconstituted chromatophores. These measurements were made at the same time as the cytochrome measurements of Fig. 1 and with the same samples, so one can compare the two figures directly. In the unextracted chromatophores (Fig. 6A), proton uptake followed each flash. *o*-Phenanthroline inhibited the proton uptake on the first flash, but only by about 50 % (Fig. 6B) (Cogdell et al. [27] have reported a similar effect of *o*-phenanthroline on *Rhodopseudomonas spheroides* chromatophores). The strong inhibitory effect of *o*-phenanthroline on the second flash is expected from its blockage of photochemistry on this flash (Fig. 1B). Extraction of Q also inhibited the proton uptake on the second flash severely, but inhibited that on the first flash by only about 50 %. The extent of

this inhibition varied among different preparations, ranging up to 75 %; but it always was less dramatic than the effect of the extraction on Y, as judged from cytochrome measurements. The proton uptake that occurred in the extracted chromatophores was not markedly sensitive to *o*-phenanthroline (Fig. 7D). Reconstitution with Q

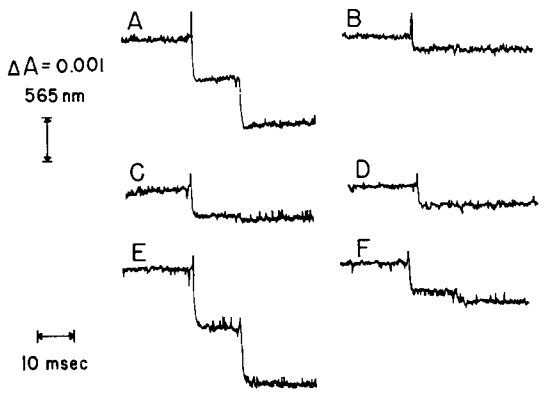

Fig. 7. Proton uptake occurring on exposure of chromatophores to two flashes. A, same sample as in Fig. 1A; B, as in Fig. 1B; C, as in Fig. 1C; D, as in Fig. 1D; E, as in Fig. 1E; and F, as in Fig. 1F. Each trace is an average of 10 measurements, with a downward deflection indicating an absorbance increase at 565 nm. Such an absorbance increase reflects a response of the phenol red indicator to a pH increase. Only negligible absorbance changes occurred in similar measurements when the sample was buffered with 0.05 M potassium phosphate at the same pH.

reversed the effects of extraction on both flashes (Figs 7E and 7F).

The rate of electron transfer from X^- to Y normally exceeds the rate of proton uptake. Measurements at pH 6.3 using bromcresol purple as an indicator have given half-times of 100–200 μs for the proton uptake [47–49], compared to about 25 μs for the secondary electron-transfer reaction (ref. 16, and our unpublished observations). Using phenol red at pH 7.0, we measured a half-time of 140 μs for the proton uptake. The kinetics of the residual proton uptake that occurred in fully extracted chromatophores were indistinguishable from the kinetics of the uptake in untreated chromatophores. This corroborates the conclusion of Chance et al. [47, 48] that the rate of the proton uptake is not closely related to the rate of the secondary electron-transfer reaction.

DISCUSSION

The present experiments show that Q or a related quinone is required for the activity of the secondary electron acceptor Y. That Q itself is Y is not a necessary corrolary, but this seems most likely in view of the abundant evidence [3, 52, 53] that illumination of cells or chromatophores can bring about the reduction of endogenous Q.

One set of observations [19] that remains difficult to interpret concerns the E_m of Y. Under certain conditions, the ability of *C. vinosum* chromatophores to perform cytochrome photooxidation on a second flash declines at low redox potentials with an apparent midpoint redox potential at pH 7 (E_{m7}) of approx. −90 mV.

But if 100 μM PMS is included in the redox buffers, titrations of secondary acceptor activity give a substantially more positive apparent E_{m7} of +70 mV. This leaves two alternatives. Either PMS must be present for a proper titration, or PMS perturbs the titration. The demonstration [19] that other components of the system (such as the low-potential cytochrome C_{552}) can be titrated readily in the absence of PMS would argue against the first of these possibilities, though perhaps not convincingly. Even if one can settle this point, a calculation of the "true" E_m from the apparent one requires additional information on the size of the Y pool [19].

The polarographic half-wave potential of Q in aqueous ethanol was reported [54] to be +126 mV at pH 7, but later workers [55] stressed factors that can complicate this measurement. In mitochondria, the E_{m7} of Q appears to be approximately +50 mV [56]. Succinate (E_{m7} = +30 mV) readily reduces much of the Q in chromatophores [53, 57–59], suggesting that the E_{m7} of their Q pool is in the same range. The apparent E_{m7} of +70 mV that one observes on titrating the secondary acceptor in the presence of PMS would be consistent with this conclusion.

It clearly would be unwarranted to assume that all of the Q in chromatophores forms a common pool. The apparent E_{m7} of −90 mV that Y exhibits under some conditions suggests that part of the Q may function in an environment that is very different from that of most of the pool. But the similarity of the responses of extracted chromatophores to Q and phylloquinone (E_{m7} reportedly −57 mV, ref. 60) indicates that the E_m of the secondary acceptor may not be critical in determining the rate or extent of the secondary electron-transfer reaction. Attempts [19] to deduce the "true" E_m of Y from its apparent E_m therefore require critical reappraisal.

The uptake of a proton that normally follows the reduction of Y would be consistent with the identification of Y as ubiquinone. But the information that is available at present does not require that the electron carriers themselves take up protons on reduction. The proton uptake could be a response of other components of the system. Additional steps in this response may account for the lag between the electron-transfer reactions and the proton uptake. An alternative simple explanation is that a barrier to the diffusion of protons separates the electron carriers from the aqueous solution.

The removal of Q has no obvious effect on the primary photochemical reaction, or on the kinetics of the back-reaction between X^- and P_{870}^+ (Figs 1–3). This argues that Q is not a component of the primary electron acceptor, but we do not consider the argument to be conclusive. P_{870} in its excited state may be sufficiently reactive that it can release electrons to a variety of acceptors, and if one disturbs the normal acceptor, a substitute may take its place. One would expect such a substitution to change the kinetics of the back-reaction, but it is conceivable that it might not. The recent observation [61–63] of the photoreduction of Q in subchromatophore preparations that have been depleted of iron illustrates the complexity of this problem.

The detailed mechanism (or mechanisms) of action of piericidin A, *o*-phenanthroline, and the alkyl-substituted quinolinequinones will require further study. Our present results do not necessarily imply that these agents act as Q analogs, and the inactivity of the alkyl-substituted benzoquinones suggests that this may not be the case. Several related benzoquinones do inhibit mitochondrial dehydrogenases [64]. 2,3-Dimethyl-5-hydroxy-6-phytyl-1,4-benzoquinone, which resembles plastoquinone

more than it does Q, does not inhibit either the secondary electron-transfer reaction in *C. vinosum* (Fig. 6), or the mitochondrial dehydrogenases [65], but it does inhibit electron flow near Photosystem II in chloroplasts [65].

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