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Structural Requirements of Quinone Coenzymes for Endogenous and Dye-Mediated Coupled Electron Transport in Bacterial Photosynthesis[1]

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Abstract

Electron transport in continuous light has been investigated in chromatophores of *Rhodopseudomonas capsulata,* Ala pho$^+$, depleted in ubiquinone-10 and subsequently reconstituted with various ubiquinone homologs and analogs. In addition the restoration of electron transport in depleted chromatophores by the artificial redox compounds *N*-methylphenazonium methosulfate and *N,N,N',N'*-tetramethyl-*p*-phenylenediamine was studied. The following pattern of activities was obtained: (1) Reconstitution of cyclic photophosphorylation with ubiquinone-10 was saturated at about 40 ubiquinone molecules per reaction center. (2) Reconstitution by ubiquinone homologs was dependent on the length of the isoprenoid side chain and the amount of residual ubiquinone in the extracted chromatophores. If two or more molecules of ubiquinone-10 per reaction center were retained, all homologs with a side chain longer than two isoprene units were as active as ubiquinone-10 in reconstitution, and the double bonds in the side chain were not required. If less than two molecules per reaction center remained, an unsaturated side chain longer than five units was necessary for full activity. Plastoquinone, α-tocopherol, and naphthoquinones

[1]Abbreviations: UQ-*n*, $n = 1$–10: ubiquinone with 1 to 10 isoprene units in the side chain; UQ-9 sat: UQ-9 with a saturated side chain; PQ: plastoquinone A; PMS: *N*-methylphenazonium methosulfate; TMPD: *N,N,N',N'*-tetramethyl-*p*-phenylenediamine; DAD: diaminodurene (2,3,5,6-tetramethyl-*p*-phenylenediamine); FCCP: carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone; E_h: redox potential; RC: photosynthetic reaction center; BChl: bacteriochlorophyll; PES: *N*-methylphenazonium ethosulfate.

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of the vitamin K series were relatively inactive in both cases. (3) All ubiquinone homologs, also ubiquinone-1 and -2, could be reduced equally well by the photosynthetic reaction center, as measured by light-induced proton binding in the presence of antimycin A and uncoupler. Plastoquinone was found to be a poor electron acceptor. (4) Photophosphorylation could be reconstituted by *N*-methylphenazonium methosulfate as well as by *N,N,N',N'*-tetramethyl-*p*-phenylenediamine in an antimycin-insensitive way, if more than two ubiquinones per reaction center remained. These compounds were active also in more extensively extracted particles reconstituted with ubiquinone-1, which itself was inactive.

Introduction

In bacterial chromatophores, as in other energy-transducing systems, quinones are the natural candidates for the role of vectorial proton carriers across the membranes. They are essential for the process of photophosphorylation [1–3] and are in considerable excess over the other redox components involved in light-dependent cyclic electron transport [1–6].

Our present knowledge on the role of UQ-10 in Rhodospirillaceae has been obtained mainly by kinetic and thermodynamic analysis of electron transport and proton binding in isolated reaction centers, native chromatophores, and UQ-extracted and reconstituted vesicles (see refs. 7–9). UQ-10 seems to perform multiple roles acting at different sites in the electron transport chain: (a) as primary acceptor (Q_A), tightly bound [10], extractable only with isooctane plus 0.1% methanol [11], and reducible in 150 psec halftime [12,13]; (b) as secondary acceptor (Q_B), more loosely bound [14], which can be reduced within about 100 μsec halftime. Proton binding is observed concomitantly with this secondary electron transfer step [15–17], yielding possibly QH^- [16,17], or QH_2 following a fast dismutation of $Q^{\cdot -}$ (see ref. 7); (c) as a large photoreducible and protonable pool, thermodynamically homogeneous ($E_{m,7} = 90$ mV) and discernible from Q_B, which is easily extractable with isooctane [3,6]; (d) as mediator of electron flow between cytochrome b_{50} and cytochrome c_2 [3,18–20]. This form of quinone seems to be identifiable with the redox carrier Z ($E_{m,7} = 150$ mV) [21, 22]; it is more tightly bound than (c) and represents only a small fraction which also is extractable with isooctane [6, 20].

While the forms of UQ in (a), (b), and (d) appear to be essential for single turnover electron transport [7, 18–20], the function of (c) and its relation with the other forms is still unclear. The differential extractability, in addition to the thermodynamic and kinetic heterogeneity, clearly suggests that exchange of UQ molecules between the different states is limited.

Removal of endogenous quinones by apolar solvents and reconstitution has been utilized as a direct approach to the understanding of the specificity required in quinone-involving reactions in various electron transport systems

(see ref. 23). However, most of these studies have been concerned only with the restoration of overall or partial electron transport activities and not with energy conservation. Attempts to reconstitute coupled electron flow have been reported only for chloroplasts; however, very low P/e^- ratios were obtained [24]. Chromatophores from photosynthetic bacteria offer the advantage of a remarkable stability of their energy coupling properties after dehydration and extraction with apolar solvents [1–3]. In particular, chromatophores from the carotenoidless mutant *Rps. capsulata,* Ala pho^+, can be lyophilized, extracted with isooctane, and reconstituted with pure UQ-10 without losing their potential ability in photophosphorylation [3]; therefore they offer a suitable system for studies on the role of UQ-10 in coupled electron flow.

This paper examines in detail the requirements in the chemical structure of UQ analogs and homologs for the reconstitution in UQ-extracted particles of the overall light-induced ATP synthesis and of partial reactions of photosynthetic electron transport. The same approach has also been utilized to investigate the sites of interaction of artificial redox mediators, PMS and TMPD, with endogenous components of the cyclic electron transport chain.

Materials and Methods

Chromatophores from the carotenoidless mutant of *Rps. capsulata,* strain Ala pho^+, were prepared, lyophilized, and extracted with isooctane as described in detail in ref. 3. The previously reported procedure was improved by freezing quickly the washed particles in liquid nitrogen before lyophilization and storing lyophilized and extracted particles under vacuum in the presence of silica gel at −80°C.

For reconstitution experiments the dried, extracted chromatophores were suspended in a small volume of isooctane and incubated for 20 min at room temperature in the dark with pure quinones, dissolved in isooctane. The reconstituted particles were dried under nitrogen and resuspended in 0.1 M glycylglycine, pH 7.4, plus 5 mM $MgCl_2$ when they were to be used for photophosphorylation experiments, or in 0.1 M KCl plus 1 mM $MgCl_2$ for proton binding measurements. All the dehydrated particles were used within a few hours after suspension in an aqueous medium. Photophosphorylation assays were performed routinely as reported in ref. 25. In some instances, and in particular always when using high concentrations of redox dyes, the measurements were carried out under controlled redox conditions; the ambient redox potential was monitored and controlled continuously in a nitrogen-flushed vessel as outlined in ref. 26. Light-induced proton binding was measured spectrophotometrically using phenol red as pH indicator at pH

7.2 following the procedure described by Takamiya and Dutton [6]. The incubation mixture containing 100 mM KCl, 1 mM $MgCl_2$, 200 μM K-ferrocyanide, 0.5 μM DAD, 10 μM FCCP, 5 μM antimycin, 50 μM phenol red, and chromatophores was placed in a spectrophotometric cuvette and kept anaerobically at a constant pH of 7.2 and E_h of 330 mV. Absorbance changes were monitored at 586–625 nm in a dual wavelength spectrophotometer; illumination was provided by an iodine-quarz lamp (55 W) focused on the cuvette through a Wratten 88 A filter. Calibration of protons was obtained by measuring the absorption changes induced by addition of standard HCl solution.

The concentration of the reaction centers was determined in the same sample from the photoinduced signal at 605–540 nm, buffering the sample with 10 mM MOPS, pH 7.0. An extinction coefficient of 29.8 mM^{-1} cm^{-1} was used [27].

The series of ubiquinones (UQ-1 to UQ-10) and plastoquinones-9 (PQ) were generous gifts from Dr. Gloor and Dr. Weber, Hoffmann-La Roche, Basel. Menaquinone, isolated from *Bacillus megaterium* (ATCC 14581), was kindly donated by Dr. A. Kröger, Munich. Phylloquinone, menadione, and DL-α-tocopherol were purchased from Merck, Darmstadt.

Determination of the total ubiquinone content was performed by following the method reported in ref. 6, except that the efficiency of recovery was routinely determined from control experiments, run in parallel, in which known amounts of pure UQ-10 were added as an internal standard.

The concentration of extracted ubiquinone was determined spectroscopically in ethanol solution utilizing an extinction coefficient of 12.5 cm^{-1} mm^{-1} at 275 nm for the oxidized minus reduced form [28].

Bacteriochlorophyll was measured in acetone–methanol extracts according to Clayton [29].

Results

Specificity of Quinones in the Reconstitution of Photophosphorylation

As reported for other photosynthetic bacteria [1–6] the UQ-10 content in chromatophores from the carotenoidless mutant of *Rps. capsulata,* strain Ala pho$^+$, depends on growth conditions and, in the case of anaerobic growth in the light, on the phase at which the cells are harvested. These variations range from 30 to 50 quinones per reaction center, increasing with the age of the culture. For these reasons the chromatophores used in the experiments reported in this paper were routinely prepared from cells harvested after 18 h of growth (inoculum about 1.5%) and contained from 35 to 42 molecules of UQ-10 per P870.

A large amount of UQ-10 can be extracted with isooctane from lyophilized chromatophores of *Rps. capsulata*, Ala pho$^+$ [3]; the amount of isooctane-extractable UQ-10 depends on the initial content present in chromatophores and on the number and duration of the extractions performed. The rate of photophosphorylation decreases in parallel with the amount of UQ-10 extracted and can be restored by incubation of depleted chromatophores with pure UQ-10 in isooctane [1–3]. This we have reinvestigated in detail. The dependence of the rate of photophosphorylation on the amount of UQ-10 incorporated is shown in Fig. 1. Saturation of the activity, at levels very close to those of lyophilized particles, is reached with amounts of UQ-10 similar to those present in unextracted chromatophores (35–42 UQ-10 per reaction center). Larger concentrations of quinone did not significantly increase the rate of photophosphorylation. To test for excess UQ-10 not incorporated, aqueous suspensions of chromatophores (2 mM glycylglycine, pH 7.2, 100 mM KCl, 2 mM $MgCl_2$) were treated by a single 30″-extraction with isooctane. Only very little UQ-10 (from 10 to 15% of the total UQ-10 added) was extracted by this technique from aqueous suspensions of chromatophores reconstituted with variable amounts of UQ-10, as described under Materials and Methods. But if the quinone was added as an ethanolic solution in small amounts to aqueous suspensions of extracted or partially reconstituted chromatophores, it was almost fully recovered in the isooctane

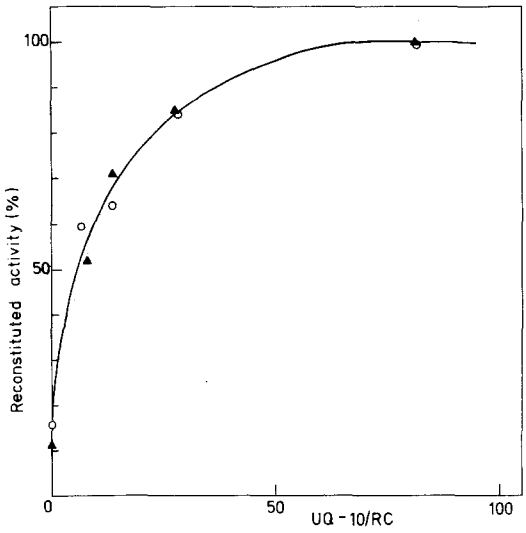

Fig. 1. Reconstitution of photophosphorylation and binding of "scalar" protons with UQ-10. The assay for photophosphorylation (open circles) and for proton binding (filled triangles) are given under Materials and Methods. 100% activity corresponds to 230 μmoles ATP formed per hour per milligram BChl and to 36 protons bound per reaction center (RC), respectively.

phase. As reported for other membrane systems [23, 29], we have found that at least 2.5-fold UQ-10 in excess over the amount originally present can be incorporated into depleted chromatophores (i.e., it was not extractable by the described procedure).

Table I summarizes a series of experiments in which the ability of different types of quinone homologs and analogs in restoring photophosphorylation was tested. The quinones were added at a constant ratio of 40 Q per reaction center. It was found that several ubiquinone homologs were able to restore photophosphorylation in depleted particles; only UQ-1 and UQ-2 were always completely ineffective. In a large number of preparations tested, two different kinds of responses were essentially found: (a) in some preparations a short-chain ubiquinone, like UQ-3, had the same effectiveness in reconstitution as that observed with UQ-10 similar to results obtained with *R. rubrum* [2]; (b) by contrast, in others, a ubiquinone homolog with a number of isoprenoid residues in the side chain higher than 5 was required for the full reconstitution of photophosphorylation. Characteristic experiments illustrating these two types of responses are reported in experiments A and B of Table I. The ability of UQ-3 to restore ATP synthesis to its maximum level was always found to be correlated to a relatively high residual activity in the depleted particles. This was also the case with UQ-9 sat, a derivative of UQ-9 with a saturated side chain. However, the reconstitution with UQ-10 reached equally high activities in both kinds of membranes, which were comparable to

Table I. Restoration of Photophosphorylation by Ubiquinone Homologs and Analogs in UQ-10-Depleted Chromatophores of *Rps. capsulata*, Ala pho^{+a}

Substance used in reconstitution	μmoles ATP formed per hour per milligram BChl	
	A	B
—	22	4
UQ-1	29	5
UQ-2	35	8
UQ-3	181	39
UQ-5	185	40
UQ-7	186	180
UQ-10	208	212
UQ-10 (+ antimycin)	0	0
PQ-9	68	23
Menadione	—	0
Phylloquinone	—	5
α-Tocopherol	—	5
Menaquinone	38	—
UQ-9 sat	183	69

[a]Extracted chromatophores were reconstituted with 40 quinone molecules per reaction center as described under Materials and Methods. The conditions of the assay can also be found there. The extracted chromatophores used in experiment A retained 2–4 molecules of ubiquinone-10 per reaction center, and the chromatophores used in B retained only 1.5 molecules per reaction center. The rate was 245 for lyophilized, unextracted chromatophores.

those measured in lyophilized chromatophores before extraction. Residual photophosphorylation activity and restoration by UQ-3 and also by UQ-9 sat in the extracted particles appeared to be related to the amount of residual coenzyme found in the different preparations. Responses of type A were observed in particles containing two or more UQ-10 per reaction center whereas at lower amounts of remaining UQ-10 (1.2–1.5 UQ per reaction center), responses of type B were found.

None of the quinone analogs tested was significantly effective in reconstitution. Plastoquinone-9, which has thermodynamic properties comparable to UQ-10 and relatively minor modifications in the structure of the benzoquinone ring, is also noticeably a poor mediator, also in particles of type A which can be restored by short-chain homologs of ubiquinone. This last result suggests that a strict structural requirement of the quinone ring has to be met for coupled electron transport in bacterial chromatophores.

In order to investigate further for which partial reaction the specificity is required, we have studied the role of various quinones at the secondary acceptor site. As suggested by Takamiya and Dutton [6], the photoreduction of quinones can be evaluated by measuring the scalar protons bound to the membrane upon illumination when cyclic electron flow is inhibited by antimycin, formation of a proton gradient is prevented by FCCP, and a nonprotonated electron-donorlike ferrocyanide for the reaction center is present. Experiments of this kind, where the bound protons were measured using the dye indicator phenol red at a pH 7.2, at an ambient redox potential of 330 mV, are shown in Fig. 2.

The small spectral signal seen in extracted particles is not eliminated by buffer and is due to a fast and reversible contribution of the reaction center.

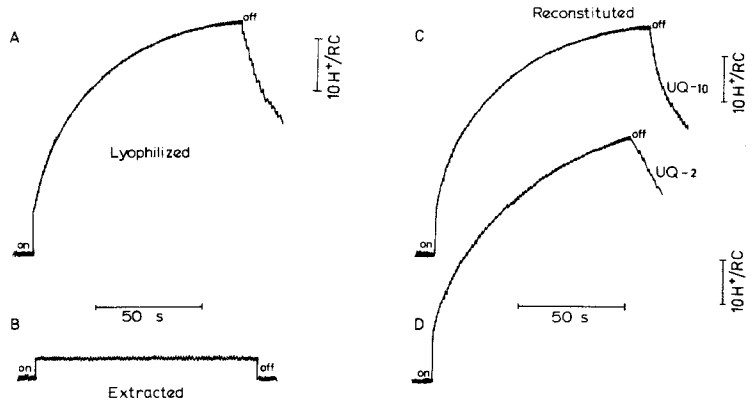

Fig. 2. Scalar proton binding in lyophilized (A), extracted (B), and reconstituted (C,D) chromatophores. The treatment of the chromatophores and the assay are described under Materials and Methods.

This contribution has always been subtracted in the computation of the amount of H^+ bound per reaction center. It is shown in Fig. 2 that proton binding is restored not only by ubiquinone-10 but also by UQ-2, which has been found to be absolutely inactive in restoring phosphorylation.

A more complete series of experiments of this type is reported in Table II. All quinones of the ubiquinone series are reducible regardless of their efficiency in reconstituting photophosphorylation. Plastoquinone is also far less effective in proton binding, indicating that the structure of the benzoquinone ring is also important for functioning as a secondary acceptor.

The relation between the extent of proton binding and of photophosphorylation, and the amount of UQ-10 incorporated in the particles, is shown in Fig. 1. Both activities are almost saturated at a ratio of UQ-10 per reaction center of about 40, which is observed in unextracted chromatophores. The saturation curves match exactly, confirming the need of a large pool of reducible quinones for high rates of photophosphorylation in continuous light.

Quinones as Redox Partners of PMS and TMPD

In a previous paper [26] we have reported that photophosphorylation in chromatophores of *Rps. capsulata,* strain Kbl, is stimulated by artificial redox dyes like PMS and TMPD. This effect was observed under continuously controlled redox conditions in anaerobiosis. At low concentrations (10 μM) of PMS or TMPD (in the presence of a mixture of other redox mediators, 2 μM each) an optimum for photophosphorylation was found at an ambient redox potential of 120 mV; at high concentrations (500 μM) of PMS or TMPD, under the same experimental conditions, the optimum for ATP

Table II. Effect of Extraction of Ubiquinone-10 and Reconstitution with Different Homologs and Analogs on Light-Induced Proton Binding, Measured in the Presence of Antimycin and Uncoupler[a]

Chromatophores	H^+/P870	QH_2/P870
Native	40	20
Lyophilized	44	22
Extracted	0	0
Reconstituted with:		
UQ-10	36	18
UQ-1	42	21
UQ-2	40	20
UQ-3	48	24
PQ-9	14	7

[a]The procedure for reconstitution (40 molecules of quinone added back per reaction center) and the assay are described under Materials and Methods. The first column gives the number of protons bound per reaction center as molar ratio, the second column gives the equivalent of hydroquinone formed per reaction center.

synthesis was shifted toward more positive potentials (200 mV). The same optimum for ATP synthesis was also observed for the reversal of antimycin inhibition by PMS and TMPD, which can be obtained at concentrations of the mediators between 100 and 500 μM.

Among the various hypotheses advanced to explain these observations we thought it likely that the interaction of artificial redox mediators with the electron transport chain could be mediated by the large quinone pool. We have tried to investigate this possibility utilizing UQ-10-depleted particles. Again two different kinds of response were obtained with respect to residual UQ-10 in particles depleted of UQ-10: (a) Some stimulation of the residual rate of photophosphorylation by PMS and TMPD, at relatively high concentrations, and the bypass of antimycin inhibition by the dyes (at 200 mV ambient redox potential) were still observable in chromatophores containing more than two UQ-10 per reaction center. (b) This phenomenon was absent, however, if the particles were more depleted in their ubiquinone complement. Examples of these two types of response are reported in Table III, experiments A and B. In both kinds of particles, preincubated in the presence of saturating amounts of UQ-10 by the usual reincorporation technique, photophosphorylation could be fully restored and presented the same response to the ambient E_h and to addition of exogenous redox mediators as unextracted preparations. If the action of PMS and TMPD is mediated via an interaction with the quinone pool, this possibility could be tested by measuring photophosphorylation in the presence of PMS and TMPD in particles into which a quinone homolog like UQ-1 or UQ-2 (see Table 1), not effective *per se* in restoring this activity but photoreducible by the primary acceptor, had been incorporated. Extracted particles, which showed no response to addition of PMS (Fig. 3), were supplemented with UQ-1 at a ratio of 40 UQ-1 per

Table III. Effect of PMS and TMPD on Photophosphorylation in Extracted Chromatophores[a]

Additions	E_h (mV)	μmoles ATP formed per hour per milligram BChl	
		A	B
Succinate (aerobic)	—	34.0	3.5
PMS (10 μM)	120	30.8	3.4
TMPD (10 μM)	120	15.0	1.5
PMS (100 μM)	200	102.7	2.4
TMPD (100 μM)	200	90.0	1.7
PMS (100 μM) + antimycin	200	94.5	2.7
TMPD (100 μM) + antimycin	200	88.5	3.2

[a]The assay was carried out under controlled redox conditions as described under Materials and Methods. In experiment A, 30 ubiquinone-10 molecules per reaction center were extracted from lyophilized chromatophores; in experiment B the value was 36. Where indicated antimycin and succinate were added to 1 and 200 μM final concentration, respectively.

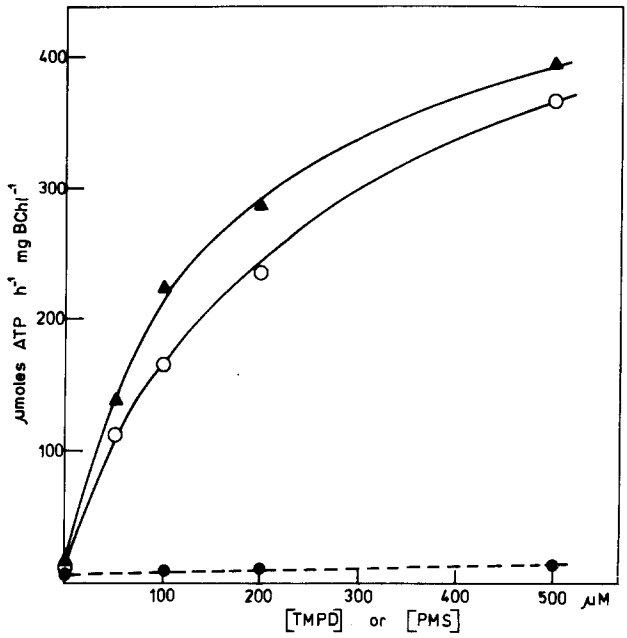

Fig. 3. Effect of UQ-1 on photophosphorylation with PMS and TMPD in extracted chromatophores. Extensively extracted chromatophores (1.7 UQ-10/RC remaining) were prepared and reconstituted with 40 UQ-1/RC as described under Materials and Methods. Filled circles represent extracted chromatophores with PMS; open circles and filled triangles represent reconstituted chromatophores with PMS and TMPD, respectively.

reaction center and their photophosphorylation activity was tested in the presence of increasing concentrations of PMS and TMPD, at a controlled ambient redox potential of 200 mV, under anaerobic conditions. As can be observed in Fig. 3, particles in which UQ-1 was incorporated show a negligible rate of ATP synthesis (see also Table I). However, this rate increases to very high values upon addition of increasing concentrations of either PMS or TMPD. These results demonstrate that the reincorporated UQ-1, which as shown in Table II can be readily photoreduced, interacts with the redox dyes reconstituting an energy-conserving electron-transport pathway. In addition they demonstrate that extensive extraction of UQ-10 (experiment B in Tables I and III) does not destroy membrane integrity and potential capability of energy conservation, since it is quite unlikely that small molecules like UQ-1 and PMS, or TMPD, could synergistically restore structural damages. Accordingly it has been reported that ion permeability is normal in extensively extracted chromatophores from *Rps. sphaeroides*, GA,

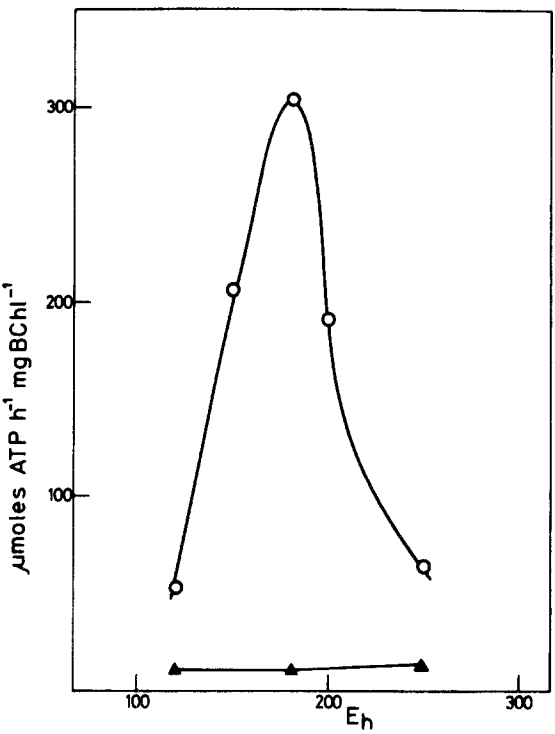

Fig. 4. E_h dependence of photophosphorylation in chromatophores reconstituted with UQ-1 and TMPD. The assay is given under Materials and Methods. TMPD was added to 200 μM (open circles). Filled triangles represent rates without TMPD. Extensively extracted chromatophores were reconstituted with 40 UQ-1/RC. In both sets of experiments the redox mediators DAD, PMS, PES, and juglone (2.5 μM each) were included in the reaction mixture.

as judged from the decay kinetics of the light-induced, field-indicating carotenoid shift [19]. In Fig. 4 it is shown that in UQ-1-particles the stimulation of photophosphorylation by TMPD (200 μM) in the presence of very low concentrations of other mediators is maximal around 200 mV; these results are analogous to those obtained in native chromatophores of *Rps. capsulata,* strain Kbl [26] and strain Ala pho$^+$ (data not shown).

As expected, antimycin had no effect in this artificial system. The reconstitution of photophosphorylation in the presence of 200 μM TMPD or PMS as a function of UQ-1 reincorporated in UQ-10-depleted vesicles is shown in Fig. 5. Saturation is achieved at a ratio of UQ-1 per reaction center of around 20–30 Q:P870, a ratio somewhat lower than that found for reconstitution of photophosphorylation by UQ-10 (cf. Fig. 1).

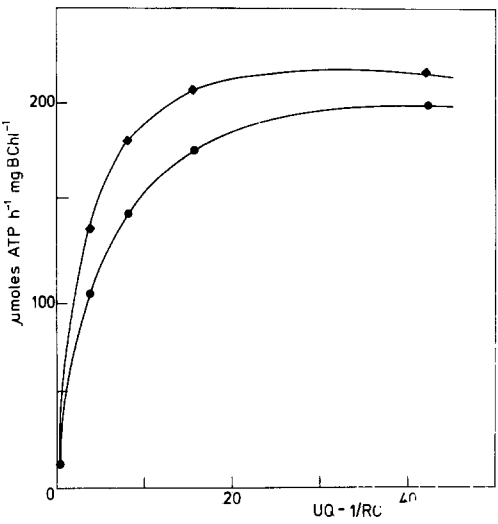

Fig. 5. Titration with UQ-1 of photophosphorylation with 200 μM PMS or TMPD in extracted chromatophores. The treatment of the chromatophores and the assay are described under Materials and Methods. Diamonds and circles represent rates with PMS and TMPD, respectively.

Discussion

The Role of the Quinone Pool

The presence of relatively large amounts of quinones in energy-transducing membranes has not been explained so far. Any mechanistic hypothesis about the redox forms involved at specific sites of the redox chains takes into account only a few quinone molecules and not the whole pool [31, 32]. Moreover, data obtained in bacterial chromatophores, a system in which electron transport can be driven by a single photochemical act, indicate that the rate of single flash-induced redox reactions is not affected by removal of the major part of the quinone complement [7]. More specifically it has been shown by Takamiya et al. [20] that a decrease in the halftime of the reduction of cytochrome c_2, after a single turnover flash at E_h of 100 mV, becomes observable only in chromatophores of *Rps. sphaeroides* extensively extracted with isooctane. Moreover, Crofts and Bowyer, evaluating the size of the pool of oxidant between the reaction center and the antimycin block in sphaeroplasts from *Rps. capsulata,* suggested that the UQ-10 involved in the cycle is kinetically distinct from bulk UQ-10 [33]. In contrast it is well established that the rate of photophosphorylation in continuous light (even when measured in a relatively short time, as 30 sec) decreases steadily with the amount of ubiquinone extracted from lyophilized chromatophores [1–3].

A second pertinent question to be resolved is the mechanism of proton carrying, transmembrane oxidoreduction reactions in biological membranes in which quinones might be involved. Arguments in favor and against a transmembrane mobility of quinones have been reviewed lately [23, 34]. An approach to the understanding of this problem has been attempted by studying the structural requirements of quinone homologs and analogs for a vectorial redox reaction in a liposome system, in particular with respect to the isoprenoid side chain [34]. There, it can be shown that the transmembrane, proton-carrying reaction of benzoquinones is considerably faster if an isoprenoid side chain of at least 15 C atoms is attached to the ring. In the present paper UQ-depleted and reconstituted vesicles from *Rps. capsulata* have been utilized for studying the role and specificity of quinones in the process of photophosphorylation. This activity, in fact, reflects directly the utilization of a transmembrane electrochemical potential difference of protons and therefore relates indirectly to the possible action of quinones as vectorial proton translocators in energy-conserving electron transport.

The results reported indicate, first, that for achieving full rates of photophosphorylation in UQ-depleted particles, the ubiquinone ring is required, plastoquinone and naphthoquinones being very poor mediators (see Table I). Second, the side chain must be formed by at least three isoprenoid units, the double bonds being not essential (experiment A in Table I). It is quite remarkable that the same requirement for the side chain has been found in the model system mentioned above [34]. In particles with a very low residual content of coenzyme Q per reaction center, however, quinones with still longer chains (more than five isoprenoid residues) and their double bonds seem to be required (experiment B in Table I). In particles from *Rps. sphaeroides,* precisely under these conditions (1.6 UQ-10 per reaction center left), it has been found that the rate of cytochrome c_2 re-reduction, measured after a flash at E_h of 100 mV, is markedly decreased [20]. On this basis the authors concluded that the carrier Z donating electrons to cytochrome c_2 could be a specialized form of ubiquinone-10 [20].

On the other hand, any ubiquinone can function as an acceptor of reducing equivalents from the reaction center (possibly via Q_A and Q_B) and can bind scalar protons independently from the length of the side chain (Table II). As discussed at length by Takamiya and Dutton [6] the indirect evaluation of the photoreduction of quinones by the proton-binding technique does not allow one to compute the absolute number of quinone molecules photoreduced, but reflects merely a steady-state situation where the leak through the antimycin-sensitive site competes with the relatively slow donation of reducing equivalents by ferrocyanide to the reaction center. Therefore this approach does not permit any quantitative evaluation of the reaction, but gives an indication of the reducibility of quinones.

Since also UQ-1 and UQ-2 are reduced and bind scalar protons, their inability to restore photophosphorylation can be related to their reoxidation step by endogenous components of the electron transport chain. The size of the protonizable pool of UQ-10 depends on the amount of UQ-10 incorporated and reaches a saturation level when about 40 quinones per reaction center are incorporated in depleted chromatophores, in spite of the fact that higher amounts of UQ-10 can be incorporated into the membrane (Fig. 1). Experiments in lyophilized chromatophores of *Rps. sphaeroides* also show a steady decrease in the extent of proton binding when the amount of UQ-10 is decreased from 30 to about 2 quinones per reaction center [6].

The saturation pattern of reconstitution suggests that there are precise kinetic or structural limitations in the size of the protonizable pool. Since the type of saturation observed for proton binding is the same for the reconstitution of the full rate of light-induced ATP synthesis (Fig. 1), the reconstitution of the secondary pool is necessary for photophosphorylation in continuous light. This apparent disagreement with single flash-induced electron flow is under further investigation.

Interaction of Ubiquinone with Artificial Redox Compounds

Artificial redox dyes, like PMS and TMPD, are capable of restoring the rate of photophosphorylation in antimycin-inhibited chromatophores. In *Rps. capsulata* this phenomenon can be observed only under specific conditions of the ambient redox potential (optimum at E_h 200 mV at pH 8.5; see ref. 26). The experiments reported in this paper show that upon extensive extraction of UQ-10 from lyophilized chromatophores, the stimulation of photophosphorylation by redox dyes in the presence or absence of antimycin disappears even at optimal E_h. On the other hand, if a certain amount of UQ-10 is retained by chromatophores, PMS and TMPD are again able to accelerate the rate of ATP synthesis. This observation indicates that in chromatophores one endogenous redox component interacting with the dyes is the UQ-10 pool.

The role of residual UQ-10 in PMS- or TMPD-mediated electron flow can be replaced by a short-chain homolog, like UQ-1 (see Fig. 4–6), which can easily be photoreduced (Table II) but cannot efficiently be reoxidized, as indicated by its inability to restore coupled electron flow (Table I). Therefore UQ-1 must act as the electron donor to the dyes. The rate of the light-induced ATP synthesis in UQ-1-reconstituted particles depends on the amount of reincorporated UQ-1 and upon the concentration of dyes present in the assay, suggesting a bimolecular collision mechanism in the quinone–dye oxidoreduction. This artificial electron flow is insensitive to antimycin.

It is noteworthy that both PMS, a proton and electron translocator, and TMPD, an electron translocator, are active in reconstituting ATP synthesis in

UQ-1 particles. This is in contrast to cyclic photophosphorylation in chloroplasts [35] and demonstrates, first, that UQ-1 is required for proton translocation through the membrane; second, that the dyes operate a redox bypass at the inner surface of the chromatophore membrane, as has been suggested earlier [26], similar to the TMPD bypass known for chloroplasts [36]; third, a long isoprenoid side chain does not seem to be required for the vectorial, proton-carrying electron transfer from the quinone pool through the membrane, but is necessary for subsequent steps in endogenous cyclic electron transport, which can be bypassed by TMPD or PMS.

By means of several criteria it has been shown that also in the liposomal model reaction mentioned above the isoprenoid chain is not required for permeation through the membrane, but facilitates quinone oxidation, in this case by ferricyanide [34]. We think that this and other similarities of the model reaction to the biological system studied here, i.e., the function of the ubiquinone pool before the antimycin-sensitive step, are not just fortuitous.

Conclusion

From this study we form the following conclusions: (1) The total, large pool of ubiquinone-10 is required for steady-state, coupled electron flow in continuous light. (2) The substituents of the quinone ring, but not the length of the isoprenoid side chain, determine the specificity as secondary electron acceptor from the photocenter. (3) A minimal length of three isoprene units in the side chain is necessary for oxidation of the quinone pool, possibly by cytochrome *b*, which leads to energization of the membrane. This specificity is abolished by *N*-methylphenazonium methosulfate or *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine. (4) A side chain longer than five units and the double bonds in the side chain are required for the function of a small, tighter bound portion of ubiquinone in the chromatophore membrane.

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