A ROLE FOR UBIQUINONE-10 IN THE $b-c_2$ SEGMENT OF THE PHOTOSYNTHETIC BACTERIAL ELECTRON TRANSPORT CHAIN

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1. Introduction

In the light-dependent cyclic electron transfer system, operating in membrane vesicles (chromatophores) from non-sulfur photosynthetic bacteria, the redox chain is vectorially arranged throughout the membranes and drives protons from the outside to the inside upon light excitation.

Following the primary photoact, which involves a transmembrane charge separation in the photochemical reaction center [1], electrons are cyclically transported through a number of redox components, the nature of which is partially known.

The involvement of ubiquinone (UQ) as a secondary acceptor of the chain has generally been accepted [2,3]; evidence has been also presented for a role of a more tightly bound form of UQ associated with iron in the primary photochemical reaction [4,5]. On kinetic grounds it has been proposed that quinone, acting as a secondary electron acceptor, is also involved in the fast light induced proton binding, detectable in chromatophores after a single turnover flash [6,3].

In *Rhodopseudomonas capsulata* chromatophores two cytochromes, one of *b* type ($E_{m,7}$ = +60 mV) and one of *c* type (cyt.c_2, $E_{m,7}$ = +340 mV) are operating in the cyclic electron flow of this system, the former accepting electrons from UQ, the latter donating them to the reaction center bacteriochlorophyll (*P*-870) [7]. From thermodynamic and kinetic evidence the presence of an additional electron carrier of unknown nature ($Z, E_{m,7}$ = +130 mV) mediating electron flow from cyt.*b* ($E_{m,7}$ = 60 mV) to cyt.c_2 has been proposed [7]. More recently, following the hypothesis proposed by Mitchell [8] on a function of UQ both on the reducing and oxidizing sides of cytochrome *b*, the possibility of a site for a ubiquinone redox couple (QH°/QH_2) has been discussed [9].

In this paper the effect of extraction and reincorporation of UQ on the redox changes of cytochromes and its relation with light-induced phosphorylation are described. The results offer a strong support for the involvment of ubiquinone in the $b-c_2$ segment of the redox chain of *Rps. capsulata*.

Abbreviation: Bchl, Bacteriochlorophyll

2. Materials and methods

Chromatophores from the carotenoid less mutant of *Rps. capsulata*, strain Ala pho$^+$, were prepared by French pressure cell breakage and differential centrifugation, as previously described [10].

The particles to be lyophilized for ubiquinone extraction were suspended in 0.05 M glycylglycine, pH 7.2, containing 5 mM $MgCl_2$ and stored overnight in ice; control particles were suspended in the same buffer in the presence of 50% glycerol (v/v) and stored at −15°C.

The procedure followed for ubiquinone extraction and reconstitution was essentially as described by Horio et al. [11]: membranes were washed with water plus 5 mM $MgCl_2$, at a concentration of about 30 μg/ml. The pellet was suspended in water at a concentration of 500 μg Bchl/ml, divided in small lots (about 2 mg Bchl each), quickly frozen in an

alcohol bath at −80°C and lyophilized overnight at 0°C. Freeze-dried samples were stored at −20°C.

For ubiquinone extraction the lyophilized chromatophores were suspended in isooctane at a Bchl concentration of about 30 μg/ml and gently shaken at 4°C for 90 min. After a low speed centrifugation the precipitate was re-extracted two or more times (see Results) for 30 min with the same procedure. The final precipitate was dried at room temperature under a stream of nitrogen. For reconstitution experiments, the extracted chromatophores were incubated at room temperature for 5–10 min with pure ubiquinone-10 dissolved in isooctane (Sigma, St. Louis). After drying, both the extracted and the reconstituted preparations were finally suspended in 0.1 M glycylglycine, pH 7.2, plus 5 mM $MgCl_2$.

Photophosphorylation assays were performed as detailed in ref. [10].

Light-induced spectral changes of cytochromes were measured under anaerobic conditions. The assays contained in final vol. 2 ml: Glycylglycine, pH 8.0, 128 μmol; $MgCl_2$, 20 μmol; Succinate 0.4 μmol; KCl, 100 μmol; and membranes corresponding to about 100 μg Bchl.

Redox changes of cytochromes were monitored with a chopped dual wavelength spectrophotometer operating at 200 cycles/s; the *RC* constant of the instrument was 2.5 ms. Optical changes were stored in a transient recorder (Datalab DL 905, Mitcham, England) and reproduced potentiometrically. Isooctane extracts were dried under vacuum and the ubiquinone content was determined spectroscopically, after dissolving the residue in spectroscopic grade ethanol, utilizing an A_{275nm} value of 12.5 cm^{-1}/mM^{-1} for the oxidized minus reduced form.

Bacteriochlorophyll was measured in acetone : methanol extracts according to Clayton [12].

3. Results and discussion

Lyophilization of water washed membranes from *Rps. capsulata* causes a decrease in photophosphorylation in the order of 50–60%. Extensive extraction of these dehydrated preparations with isooctane results in a progressive depletion of ubiquinone (in these membranes only UQ-10), which is recovered in a practically pure form in the supernatants (fig.1). The amount of isooctane extractable UQ, on a Bacteriochlorophyll basis, is somewhat variable from preparations to preparations, from 0.7–1.3 μmol/mg Bacteriochlorophyll (compare also [11]) and is probably related to the growth phase of the culture. If these values are compared with the cytochrome content in the same membranes this would correspond to an excess of 15–25 fold of UQ over the total dithionite reducible cyt.*b* (see also [13]). In relation to the content of UQ in the membranes a variable number of extractions (from a minimum of two to a maximum of five) is needed in order to obtain a drastic reduction in the rate of light-induced phosphorylation (for an example see fig.2).

Reincorporation into the membrane of pure UQ-10 amounts corresponding approximatively to those extracted with isooctane, can restore photophosphorylation almost to the level of lyophilized unextracted vesicles.

In native chromatophores cyclic electron flow and associated phosphorylation are completely inhibited by Antimycin A, an inhibition believed to block electron flow between cyt. *b* and cyt.c_2. This

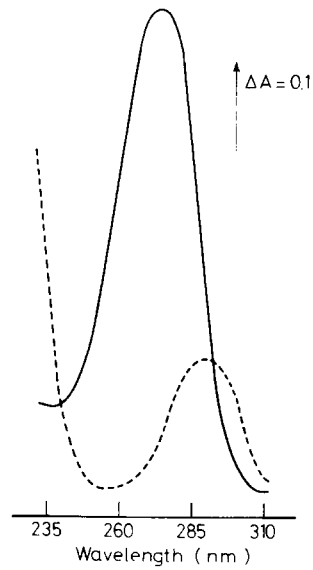

Fig.1. Absorption spectra of the ethanol dissolved residue of isooctane extracts from chromatophores of the carotenoidless strain Ala pho$^+$ of *Rps. capsulata*. Continuous line: oxidized, no additions; broken line: reduced with KBH_4.

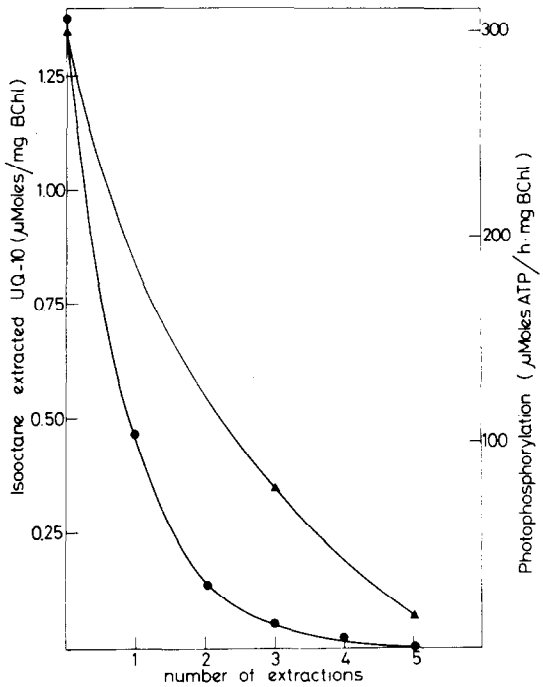

Fig.2. Correlation between the amount of UQ extracted from bacterial membrane fragments and the rate of photophosphorylation catalyzed by UQ-depleted chromatophores.

inhibition can be completely relieved by phenazine methosulfate (PMS) (table 1) under suitable redox conditions (A. Baccarini-Melandri, in preparation). In table 1 the sensitivity of photophosphorylation activity in the UQ-reconstituted system to inhibition by antimycin A and the bypass of this block by PMS are also documented; the data demonstrate that reincorporation of UQ-10 leads to a restoration of photophosphorylation linked to the flow of electrons through the b–c_2 segment of the cyclic electron transport chain. Similar results were previously reported in *Rhodospirillum rubrum* chromatophores [13].

The effect of UQ extraction and reincorporation on the redox changes of cytochromes induced by continuous light, was also examined. In these experiments no effort was made to control the ambient redox potential by means of redox buffers and mediating dyes in order to avoid any possible bypass reaction in the natural electron transfer pathway by the redox mediators. Na-succinate, 200 μM, was added to the anaerobic assay mixture: these conditions are known to poise the redox potential close to the optimum for cyclic electron flow.

In lyophilized chromatophores, upon illumination, a fast reduction of cyt.*b* (fig.3) is readily observed which is rapidly reversed in the dark. The kinetics of both the photoreduction and reoxidation reactions are close to, or more rapid than, the time constant of the chopped dual wavelength spectrophotometer used and are qualitatively similar to those of native unlyophilized preparations.

Addition of antimycin A stimulates by several folds the extent of photoreduction of cyt.*b* and practically blocks its reoxidation (a slow reoxidation of cyt.*b* was however observed under aerobic conditions).

Repetitive extractions of UQ markedly affects these light-dependent redox changes of cyt.*b*: a slower photoreduction of cyt.*b* can be observed, in line with the proposed role of UQ on the reducing side of this

Table 1
Effect of UQ-depletion and UQ-reconstitution on photophosphorylation in chromatophores of *Rhodopseudomonas capsulata*, Ala pho$^+$

Additions	Untreated	Lyophilized	Extracted	Reconstituted
	(μmol ATP formed/h·mg Bacteriochlorophyll)			
None	804	296	14	286
PMS 100 μM	1362	409	68	492
Antimycin 4 μM	2	0	0	0
Antimycin 4 μM and PMS 100 μM	675	273	n.d.	432

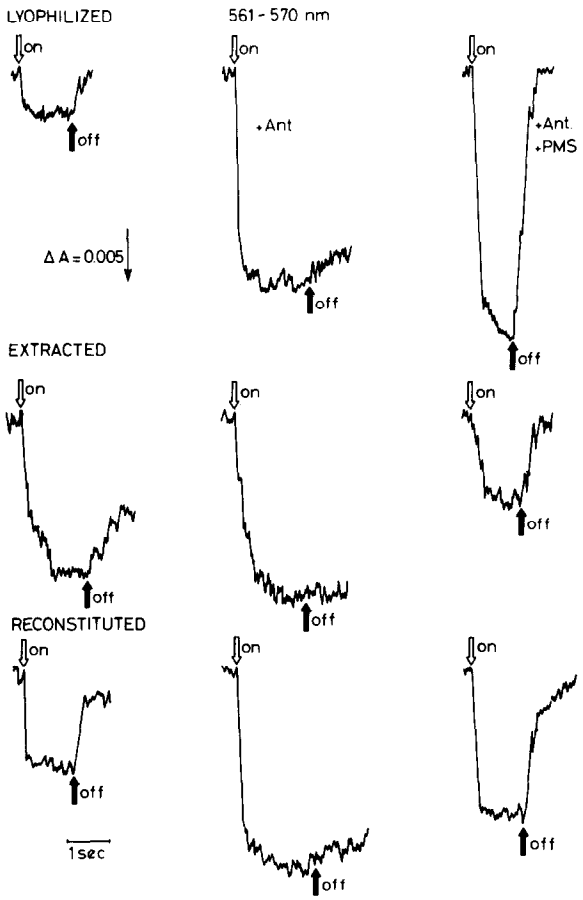

Fig.3. Redox changes of cyt.*b* induced by continuous illumination in lyophilized, isooctane extracted and UQ-10 reconstituted chromatophores. Lyophilized membranes: 65 μg Bchl/ml; extracted and reconstituted membranes: 52 μg Bchl/ml. Additions: Antimycin A, 4 μM; PMS, 100 μM.

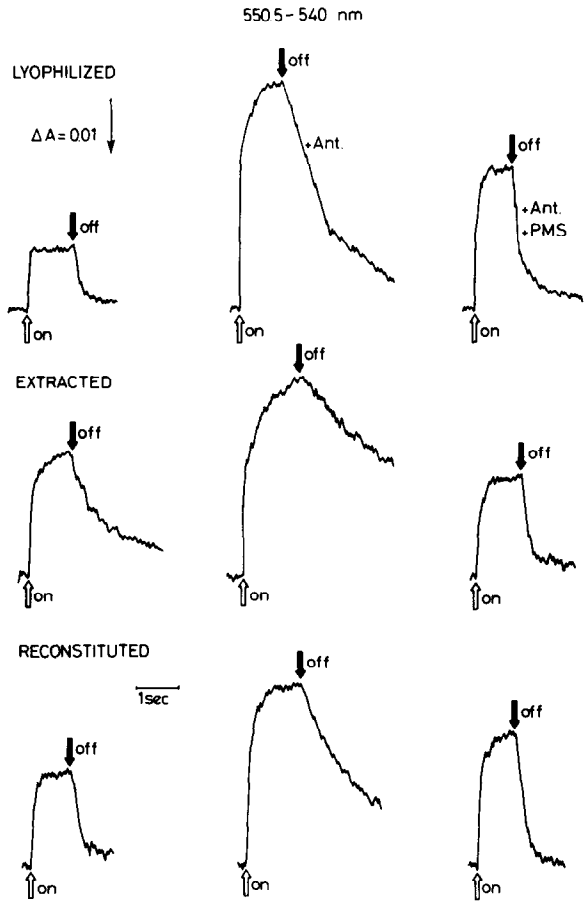

Fig.4. Redox changes of cyt.*c*-induced by continuous illumination in lyophilized, isooctane extracted and UQ-10 reconstituted chromatophores. Conditions as in fig.3.

carrier; however a very significant decrease also in the rate of reoxidation in the dark after illumination can be detected. The decrease in the rate of cyt.*b* reoxidation in the dark in extracted particles suggests a role of UQ also on the oxidizing side of cyt.*b* in the cyclic electron transfer. This conclusion is very strongly supported by the lack of any substantial crossover effect on the steady state reduction level of cyt.*b* induced in UQ-depleted particles by antimycin A. In these preparations and in the absence of antimycin A, cyt.*b* reaches levels comparable to those observed in the presence of this inhibitor in lyophlized and reconstituted membranes.

Reincorporation of UQ-10 into the extracted membranes restores fast rates of photoreduction and reoxidation of cyt.*b* in the dark; antimycin A has qualitatively the same effect observed in control particles (fig.3). These observations are particularly important if considered in connection with the reconstitution of photophosphorylation by UQ-10, since they demonstrate that the electron transfer pathway in extracted membranes, incubated with exogenous UQ-10, involves an antimycin sensitive site and is coupled to energy transduction. This strongly suggests that exogenous UQ-10 restores the electron transfer reactions operating in native membranes.

Symmetrical effects of extraction and reincorporation of UQ-10 on the photoinduced redox changes of cyt.c_2 are shown in fig.4. Following UQ depletion a very significant decrease in the rate of reduction in the dark is observed; this is accompanied by a decrease in the stimulation of the steady level of photooxidation induced by antimycin A. The fast redox changes characteristic of native or lyophilized control particles are fully restored upon reconstitution with UQ-10. The difference in the effect of antimycin addition on the kinetic of cyt.*b* oxidation, which is practically blocked by the inhibitor and of cyt.c_2 reduction, which is only partially inhibited, can also be seen. These results, obtained under continuous light with a relatively slow responding apparatus, may suggest that, under anaerobic conditions, an antimycin insensitive electron flow not involving cyt.*b* exists [15,16].

The effect of PMS in bypassing the antimycin inhibition of cyclic electron flow deserves some comments. PMS (100 μM) appears to catalyze a fast reoxidation of cyt.*b* and reduction of cyt.c_2 in antimycin-inhibited, lyophilized or reconstituted chromatophores; however while with PMS plus antimycin the steady extent of cyt.*b* photoreduction is not lower than that in the presence of antimycin alone, the level of cyt.c_2 photooxidation is decreased. This indicates that although PMS is a relatively good mediator of electron transport between cyt.*b* and cyt.c_2, the rate of the PMS catalyzed reactions is slower that that of the endogenous cycle especially at the step of cyt.*b* oxidation [17]. Upon UQ extraction the effect of the pattern of PMS action is drastically altered: in this case PMS lowers markedly also the steady state extent of cyt.*b* photoreduction as compared with that of unihibited or antimycin-supplemented membranes. This demonstrates that, in the presence of PMS, the effect of the depletion of UQ is observed specifically on the reducing side of cyt.*b* and that in UQ-depleted particles, PMS is still an oxidant for cyt.*b* and an excellent electron donor for cyt.c_2 [17]. PMS therefore appears to catalyze an antimycin insensitive cyclic electron flow, accepting electrons from cyt.*b* and donating them to cyt.c_2 with a rather specific mechanism ([18] but cf. [19]).

It is known that the properties of cytochromes of *b* type may be sensitive to the hydrophobic environment of the membrane (e.g. [20,21]); it is therefore possible, in principle, that extraction of UQ with apolar solvents may cause functional alterations of

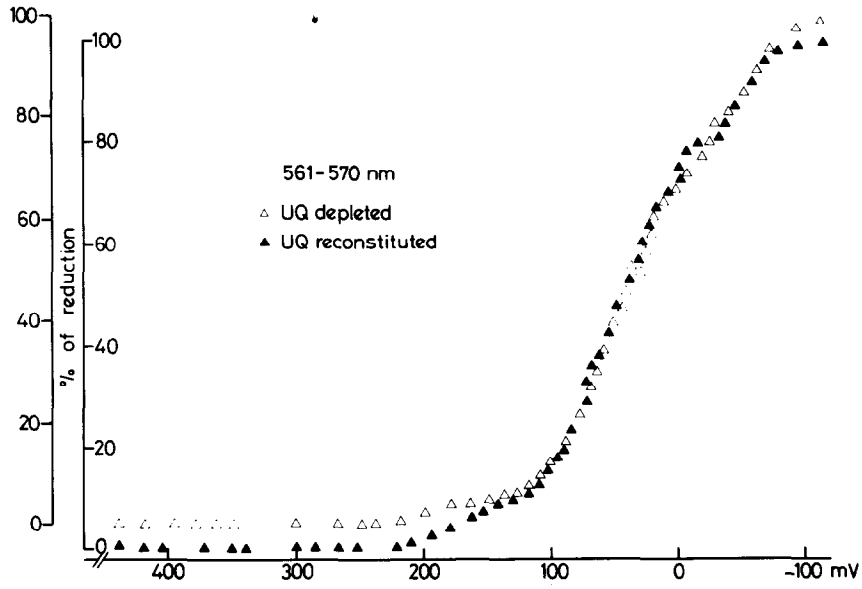

Fig.5. Redox titrations at 561–570 nm of isooctane extracted and UQ-10 reconstituted chromatophores. The experiments were performed as outlined in [27].

cytochromes *b*, which could be reversed by UQ-10 reincorporation. However, redox titrations of cyt.*b* in extracted and reconstituted chromatophores (fig.5) show no change in the 'in situ' thermodynamic properties of these carriers. The response of cytochromes of *b* type to the ambient redox potentional is quite similar to that observed in native untreated chromatophores (Zannoni, D., unpublished observations; see also [22]).

The results presented in this paper support the concept of an involvement of the UQ pool, (particularly large in this type of membrane, 0.8–1.4 mol/mol Bchl) which is more complex than that of merely a secondary electron acceptor. These data can be related to experiments on the respiratory chain of UQ-deficient mutants of *Escherichia coli* [23], on the inhibition of electron transfer by the ubiquinone analog dibromothymoquinone in *Rhs. rubrum* chromatophores [15] and in rat heart mitochondria [24], and on the redox reactions of UQ-extracted mitochondrial membrane fragments [25]. The complexity of the cyt.*b*–UQ interaction in all electron transfer chains, as indicated by the above mentioned and many others studies, has been recently rationalized by P. Mitchell [26] in the hypothetical scheme of the '*Q* cycle'.

The remarkable stability to UQ extraction and reinsertion of the photosynthetic bacterial membranes used in the experiments described in this paper offers an experimental system quite suitable for investigating the problem of cytochrome *b*–ubiquinone interaction. Experiments by single turnover flash spectrophotometry on this sytem are now in progress.

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