A SPECIFIC ROLE FOR RHODOQUINONE IN THE PHOTOSYNTHETIC ELECTRON-TRANSFER SYSTEM OF *RHODOSPIRILLUM RUBRUM*

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1. Introduction

Quinones are essential constituents of the electron-transfer systems which mediate energy conversion in biological membranes. The functional versatility of these lipophilic redox agents is particularly apparent in bacterial photosynthesis, where quinones appear to participate in at least 4 distinct redox steps [1]. In the facultative photoheterotroph *Rhodospirillum rubrum*, all those functions have been ascribed to ubiquinone-10 [2] whereas no specific participation in photochemical electron transfer has been demonstrated for rhodoquinone-10, the other quinone detected in the membrane system of that bacterium [3]. Here we describe the characterization of a non-phototrophic *R. rubrum* mutant which lacks rhodoquinone. The properties of this strain indicate that rhodoquinone is specifically required for *R. rubrum* photosynthesis as a constituent of the electron-transfer side chain which is apparently involved in the redox regulation of the main cyclic pathway [4–6].

2. Methods

Wild-type *R. rubrum* (strain S1), its non-phototrophic mutant derivative (strain F11) and the phototrophic revertant of the mutant (strain RF110) were described in [7]. Cultures were performed in the dark under low oxygen tension [6] to derepress pigment synthesis [8]. Photosynthetic membrane vesicles (chromatophores) were prepared as in [9]. Analysis of the benzoquinone levels of lyophilized chromatophores was achieved by extraction with acetone–methanol and thin-layer chromatography of the extracts on silica gel plates [10]. The relative intensity of the spots was measured with a Vernon photometer (PHI-3). Purified ubiquinone and rhodoquinone were obtained from lyophilized wild-type cells as in [11]. Light-dependent oxygen uptake was estimated as in [12], using 50 μM reduced 2,6-dichlorophenolindophenol as the terminal electron donor and 45 mM sodium phosphate (pH 7.4) as the buffer. Photoreduction of tetrazolium blue and methyl red were monitored with a Hitachi-356 spectrophotometer, following the light-dependent ΔA at 590 and 443 nm, respectively. The reaction mixtures, in cuvettes of 1 cm optical path, contained: 45 mM sodium phosphate (pH 7.4), 50 μM 2,6-dichlorophenolindophenol, 1.6 mM sodium ascorbate; 50 mM glucose; 9 units/ml glucose oxidase (EC 1.1.3.4), 400 units/ml catalase (EC 1.11.1.6), 3.6 μM bacteriochlorophyll (as chromatophores) and 50 μM tetrazolium blue or methyl red, as required. The mixture was covered with 0.5 ml paraffin oil to minimize oxygen diffusion. Actinic illumination was as in [6].

3. Results

Rhodospirillum rubrum strain F11 is a mutant unable to grow under phototrophic conditions [7]. Chromatophores isolated from strain F11 sustain normal rates of cyclic photophosphorylation but are deficient in photooxidase activity, that is, in the ability to catalyze the light-dependent transfer of electrons from exogenous donors to oxygen and to artificial acceptors such as tetrazolium blue and methyl red (fig.1) [7]. This work was aimed to identify the endogenous chromatophore constituent(s) which is (are)

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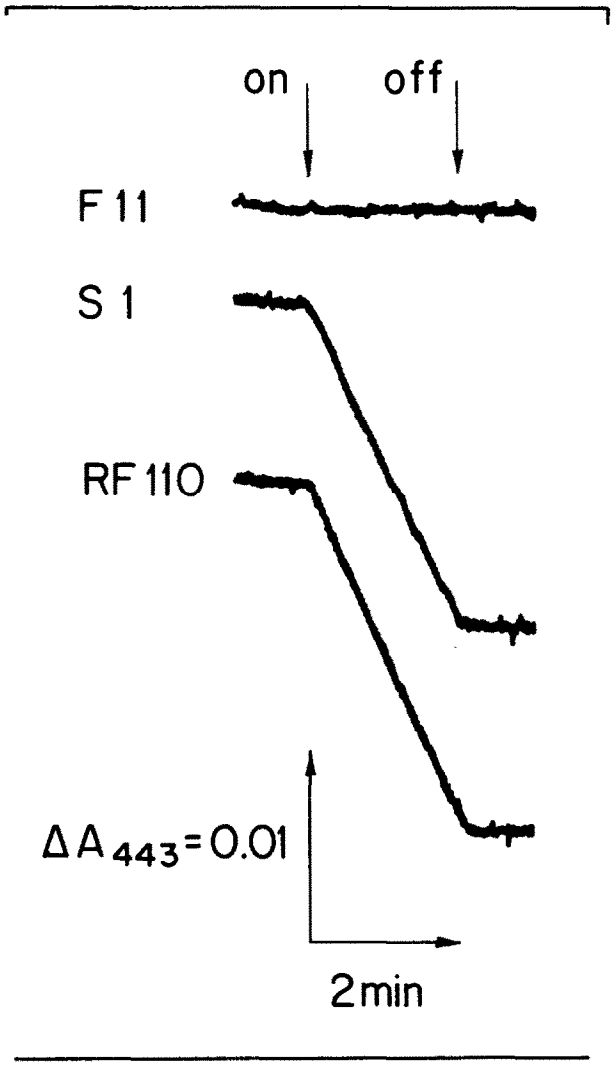

Fig.1. Photooxidase activity of lyophilized chromatophores with methyl red as the terminal electron acceptor.

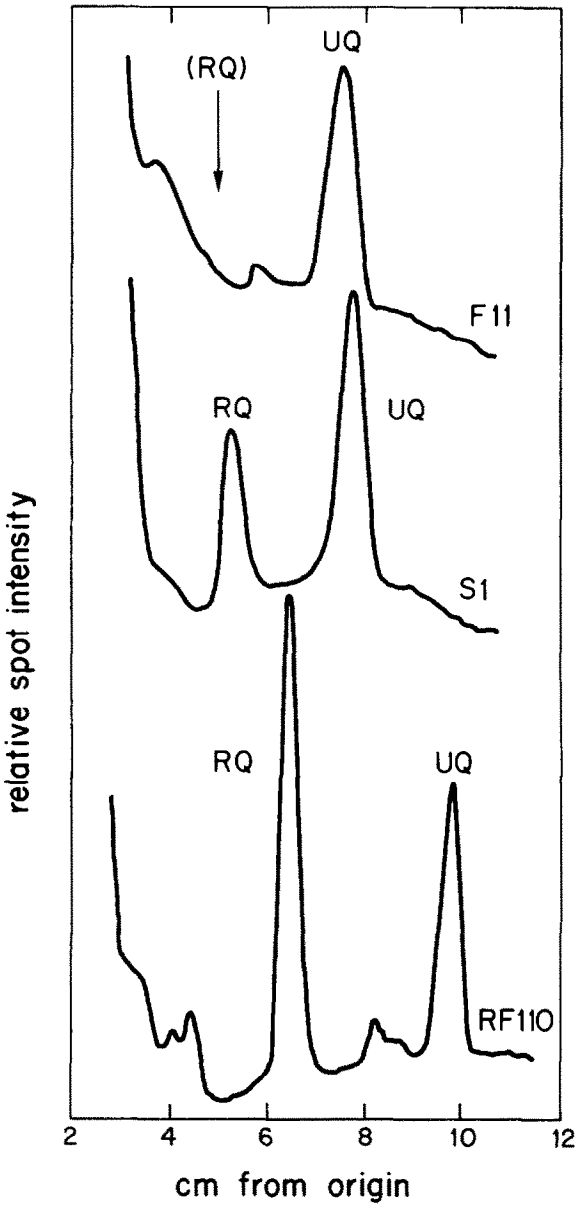

Fig.2. Silica gel thin-layer chromatography of benzoquinones extracted from lyophilized chromatophores from strains S1, F11 and RF110. Ubiquinone (UQ) and rhodoquinone (RQ) were identified both by co-chromatography of the extracts with purified quinones and by the spectral properties of the spot eluates. The arrow indicates the expected position of the missing rhodoquinone spot in the F11 chromatogram as estimated from the distance travelled by rhodoquinone relative to that travelled by ubiquinone when both were present (R_{UQ} 0.65).

altered in the mutant as a means to characterize the system which is responsible for photooxidase activity in *R. rubrum*.

Analysis of the acetone–methanol extracts of lyophilized chromatophores by thin layer chromatography shows that strain F11 lacks detectable levels of rhodoquinone while its ubiquinone content is similar to that of the parental strain, S1 (fig.2). Since ubiquinone seems to be a metabolic intermediate of rhodoquinone biosynthesis [11], it is likely that the mutation carried by strain F11 affects one, at least, of the enzymes which mediate the conversion of

ubiquinone into rhodoquinone.

Both photooxidase activity and rhodoquinone are simultaneously recovered by spontaneous revertants which were selected from strain F11 only for restoration of phototrophic growth. Those observations, which are illustrated in fig.1,2 for revertant strain RF110, indicate that rhodoquinone is a constituent of the photooxidase system and also that it is required for normal photosynthesis in *R. rubrum*.

Additional evidence supporting the participation of rhodoquinone in photooxidase activity is provided by the results of experiments in which we attempted to reconstitute the catalytic activity by adding purified quinones to mutant chromatophores. With any of the terminal electron acceptors used, rhodoquinone elicited a marked stimulation of the activity while no enhancement was caused by ubiquinone, the major benzoquinone of *R. rubrum* membranes (fig.3).

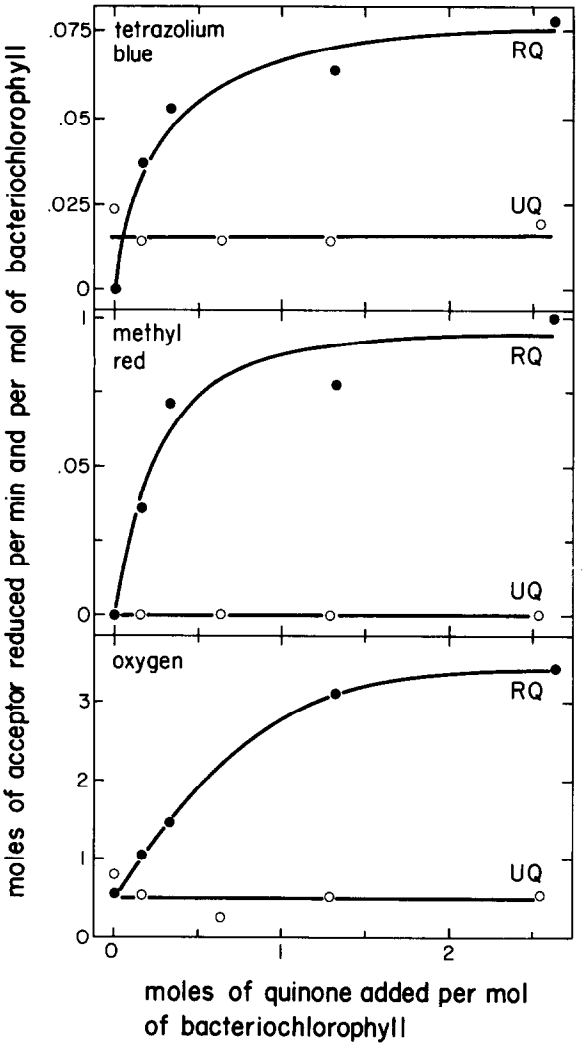

Fig.3. Effect of rhodoquinone (RQ) and ubiquinone (UQ) on the photooxidase activity of strain F11 chromatophores. Purified quinones were added in isooctane solution to lyophilized chromatophores as in [13].

4. Discussion

The photooxidase system consists of a part of the cyclic chain of electron carriers, which includes at least the photoreaction center, and of a specific side chain, which connects the low-potential cyclic acceptors to oxygen and which is defective in strain F11 [4]. If rhodoquinone does not participate in cyclic electron transfer, as it appears from the high phosphorylative activity of strain F11 chromatophores [7] and from its ineffectiveness (as compared to ubiquinone) in restoring cyclic photophosphorylation in wild-type chromatophores extracted with organic solvents [13], the involvement of this quinone in photooxidase activity suggests that it is a constituent (or perhaps the only one) of the specific photooxidase side chain. Furthermore, as reduced rhodoquinone is highly autooxidizable [11], this redox carrier appears to be a plausible candidate for the endogenous constituent which reacts directly with oxygen in the light-dependent process.

Experiments done mainly with intact cells [5,6] indicated that the redox interaction between cytoplasmic reductants and the components of the cyclic system may lead to an overreduction of the cyclic acceptors which eventually results in the inhibition of cyclic photophosphorylation. We concluded [5,6] that the physiological function of the photooxidase side chain is that of draining away the electrons in excess by transferring them to oxygen and, presumably, to other cytoplasmic acceptors. According to that hypothesis, the identification of rhodoquinone as a constituent of the side chain would explain why this redox agent is required by *R. rubrum* photosynthesis. However, the possibility that rhodoquinone has some other roles in the electron-transfer systems of *R. rubrum* should not be discarded. Ubiquinone participates in several distinct redox steps of both respiration and photosynthesis in the same organism [1]. The rhodoquinone-deficient strain may be very useful to explore that possibility.

Finally, it should be remarked here that these data

(fig.3) do not rule out the participation of ubiquinone in photooxidase activity because this benzoquinone is present at normal levels in mutant chromatophores (fig.2). To the contrary, ubiquinone is likely to be one of the carriers which are shared by the cyclic and the photooxidase systems, as expected from its role as the primary, metastable acceptor in the photoreaction center of *R. rubrum* [1].

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