

Transfer of ubiquinol from the reaction center to the bc_1 complex in *Rhodobacter sphaeroides* chromatophores under oxidizing conditions

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The mechanism of interaction between the photosynthetic reaction center (RC) and bc_1 complex has been investigated in chromatophores of *Rhodobacter sphaeroides*. The kinetics of cytochrome b_h reduction and formation of the transmembrane electric potential were measured at high E_h , a condition where ubiquinol is formed in the RC only on the second light flash. In the presence of antimycin A, the kinetics of cytochrome b_h reduction have been shown to be sensitive neither to the amount of ubiquinol produced nor to the number of active bc_1 complexes. It is concluded that the reaction between the ubiquinol produced on the second flash and the bc_1 complex is monomolecular. To explain the monomolecular pattern of this reaction under oxidizing conditions (the present work) and the previously described bimolecular pattern under reducing conditions [(1983) *Biochim. Biophys. Acta* 723, 202–218], it is proposed that (i) quinone exchange between the RC and bc_1 complex occurs via a local quinone pool and (ii) the rate of exchange between the quinone pools is very much slower than cytochrome b_h reduction.

Reaction center; Ubiquinol–cytochrome-*c* reductase; Electron transfer; Ubiquinol; (*Rhodobacter sphaeroides*)

1. INTRODUCTION

In chromatophores of non-sulphur purple bacteria, the cyclic electron-transport chain consists of a photosynthetic reaction center and bc_1 complex. Following exposure to light flashes, the former reduces the ubiquinone and the latter oxidizes ubiquinol [1,2]. When a ubiquinol molecule is oxidized by the oxidase site Z of the bc_1

complex, one electron is transferred via a sequence of carriers to the donor side of the RC to reduce P870⁺ and one electron is passed via hemes b_l and b_h to the reductase site C to reduce ubiquinone.

The mechanism of ubiquinol transfer from the RC to the bc_1 complex is still unclear. Crofts and co-workers [3–6] have shown that when the pool is reduced at equilibrium, the interaction between the ubiquinol and bc_1 complex occurs via a bimolecular mechanism. O'Keefe and co-workers [7], however, have postulated direct interaction between the RC and bc_1 complex.

The purpose of the present work is to investigate further the mechanism of ubiquinol transfer from the RC to the bc_1 complex.

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Abbreviations: RC, reaction center; P870, reaction center bacteriochlorophyll dimer; Q_A, Q_B, primary and secondary quinone acceptors of RC; $\Delta\psi$, transmembrane electric potential difference; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine; bc_1 complex, ubiquinol:cytochrome c_2 oxidoreductase; b_l, b_h , low- and high-potential cytochrome *b* hemes of bc_1 complex (*b*-566 and *b*-561)

2. MATERIALS AND METHODS

Cells of *Rhodobacter sphaeroides* (wild-type, strain R-1) were grown and chromatophores isolated by French press treatment

as described [8]. Measurements of $\Delta\psi$ generation and absorption changes were conducted as in [9,10]. Kinetic curves were analyzed using a modification of the DISCRETE program [11].

Q_B function in collodion film-associated chromatophores was reconstituted by adding Q-10 (20 mg/ml) to the solution of asolectin in decane used to impregnate the collodion film in the measuring cell, as described in [12].

3. RESULTS

In *R. sphaeroides* chromatophores, following saturating light flashes, the formation of the semiquinone species Q_B^- is observed to oscillate with even-numbered flashes [13,14]. In dark-adapted preparations, a bound semiquinone Q_B^- is formed on the first flash and ubiquinol on the second [1,15-17]. The ubiquinol leaving the RC is oxidized by the bc_1 complex. When the quinone pool has been oxidized prior to the flash, both the extent of reduction of cytochrome b_h and $\Delta\psi$ generation oscillate with flash number [7,8,13,18,19]. In fig.1 we present on two time scales the pattern of $\Delta\psi$ formation in *R. sphaeroides* chromatophore membranes following the first and second flash. Subsequently to the first flash, one observes a rapid rise of $\Delta\psi$ ($\tau < 0.1 \mu s$), due to charge separation between P870 and Q_A [20], followed by a small phase associated with the reduction of $P870^+$ by cytochrome c_2 [21] and then by the slow decay due to passive charge leakage [20]. Following the

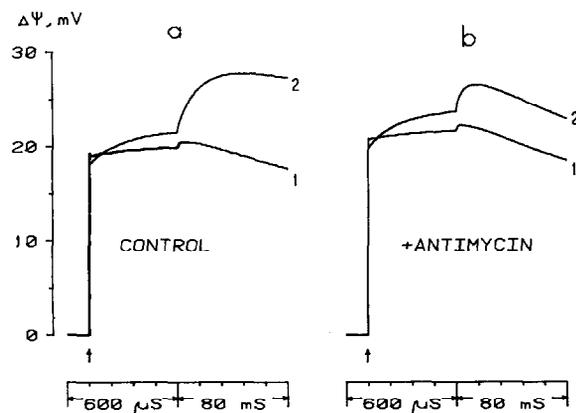


Fig.1. Generation of $\Delta\psi$ in *R. sphaeroides* chromatophores following the first (1) and second (2) laser flashes in the absence of inhibitors (a) and in the presence of $4 \mu M$ antimycin A (b). The incubation medium contained: 30 mM HEPES (pH 7.5), 1 mM potassium ferrocyanide, 30 μM TMPD. Arrows show flashes. Time lag between the 1st and 2nd flash, 2 s; $E_h = 300$ mV.

second flash, at least two more phases appear, one being fast ($\tau \sim 0.15$ ms) and the other slow ($\tau \sim 20$ ms) (curve 2). The fast phase is due to electrogenic protonation of the secondary acceptor [12,22], the slow phase being due to electrogenic reactions in the bc_1 complex [8]. Antimycin A has no effect on the fast phase but partially suppresses and accelerates the slow phase (fig.1b) by blocking both electrogenic reactions at the ubiquinone reductase site and further turnovers of the bc_1 complex [8]. The millisecond phase of $\Delta\psi$ formation observed after the addition of antimycin A arises from the oxidation of ubiquinol and reduc-

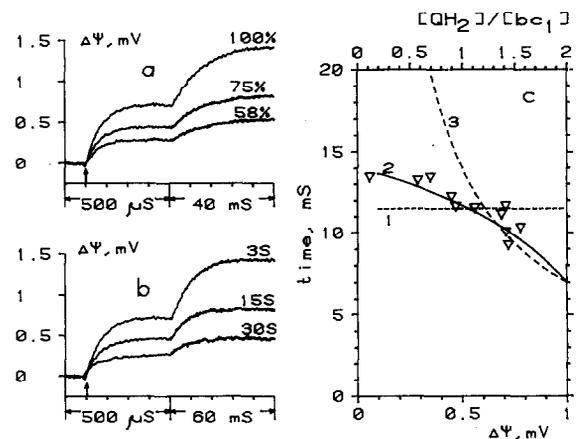


Fig.2. The difference of photoelectric responses of *R. sphaeroides* chromatophores induced by the 2nd and 1st flash (b). (c) Rise time of the antimycin-sensitive phase of $\Delta\psi$ formation as a function of the amplitude of the Q_B^- protonation phase (lower axis) and of the relative amount of QH_2 formed after the 2nd flash (upper axis, see also explanations to the equation below). The incubation medium contained: 30 mM Mes (pH 6.1), 2 mM ferrocyanide, 50 μM TMPD, 4 μM antimycin A, 1 μM methylene blue. Theoretical curves: bimolecular interaction between ubiquinol and bc_1 complex (curve 3); supercomplex formed by the RC and bc_1 complex (curve 1); interaction between the RC and bc_1 complex via a local quinone pool (curve 2). In the latter case, the time of reduction of cytochrome b_h (T) depends on the probability α of ubiquinol formation after the second flash. It can therefore be calculated using the following approximate formula:

$$T(\alpha) = \frac{\alpha^2 \tau / 2 + 2\alpha(1 - \alpha)\tau}{\alpha^2 + 2\alpha(1 - \alpha)} = \frac{\tau(4 - 3\alpha)}{4 - 2\alpha}$$

where τ is the time of reduction of cytochrome b_h when only one ubiquinol is available in the local pool. For theoretical calculation τ was taken to be 14 ms. The maximal value of α under our conditions was estimated as 0.8 from the model described in [2].

tion of the b_h heme. It is completely suppressed by myxothiazol [8].

The amplitude of the 0.15-ms phase appears to be proportional to the amount of ubiquinol produced on the second flash. The amplitude of the antimycin-insensitive 10-ms phase is proportional to the population of reacting bc_1 complexes. Thus, the behaviour pattern of this single kinetic curve reflects events occurring in both the RC and bc_1 complex.

To elucidate the interaction between the RC and bc_1 complex, the formation of the RC ubiquinol was varied in two ways: by decreasing the intensity of the second flash (using light filters) (fig.2a) or by increasing the dark time lag between the first and second flash (to cause Q_B^- oxidation in the dark by a mediator) (fig.2b).

In the case of a bimolecular character of interaction between the ubiquinol expelled from the RC and the bc_1 complex, the rate of $\Delta\psi$ formation by bc_1 complex would decrease essentially with reduction in the amounts of ubiquinol. Fig.2c shows the rise time (τ) of the electrogenic phase related to the bc_1 complex in the presence of antimycin A as a function of the amplitude of the fast (microsecond) electrogenic phase. One can see that a 10-fold decrease in the amount of ubiquinol produced causes a negligible increase in the time of $\Delta\psi$ generation. Over the range pH 6–8.5, the above dependence has a similar behaviour pattern.

The study was continued by measuring the kinetics of flash-induced reduction of cytochrome b_h in the presence of antimycin A. Kinetic curves

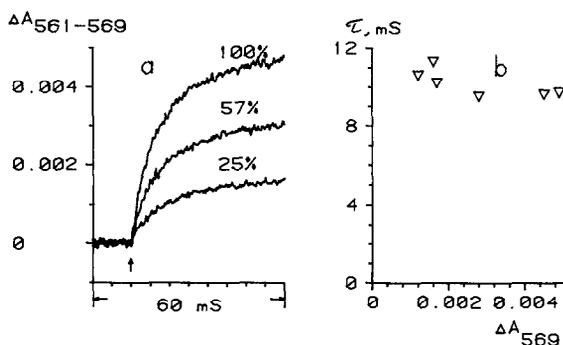


Fig.3. Effect of flash intensity on cytochrome b_h reduction (a) and on its reduction time (b). Percentage of flash intensity given beside each curve. Abscissa in (b): 569 nm absorption changes (proportional to the extent of flash-induced oxidation of P870). Incubation medium as in fig.1. Each curve was derived by averaging 16 curves.

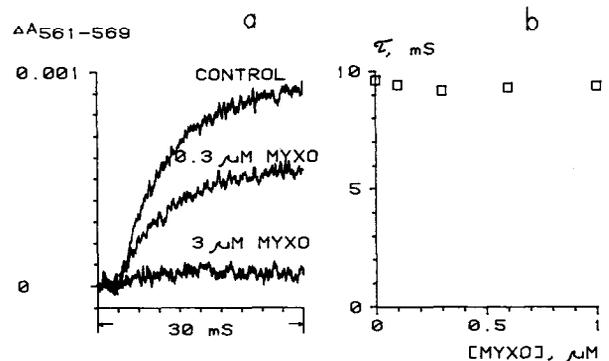


Fig.4. Effect of myxothiazol on photo-induced reduction of cytochrome b_h (a) and on the time of its reduction (b). Incubation medium as in fig.1. (1) Control, (2) 0.3 μM myxothiazol, (3) 3 μM myxothiazol. Each curve was derived by averaging 16 curves.

of cytochrome b_h reduction for different flash intensities are shown in fig.3a. Due to the effect of the measuring beam (for details see [17]), the Q_B^- is present in about half of the RCs. Consequently, one ubiquinol molecule per bc_1 complex is formed after each saturating flash. As the intensity of the flashes is decreased, the amount of reduced cytochrome b_h decreases (fig.3a), its reduction time remaining practically unchanged (fig.3b).

In the case of a bimolecular pattern of the reaction between the ubiquinol and bc_1 complex, its rate would decrease with reduction of both the ubiquinol available and the amount of active bc_1 complexes. Data from an experiment in which the amount of active bc_1 complexes was varied using myxothiazol are shown in fig.4. As demonstrated, the addition of myxothiazol causes a decrease in the reduced cytochrome b_h population (fig.4a) but does not affect the rate of its reduction (fig.4b).

4. DISCUSSION

A bimolecular mechanism of interaction between ubiquinol and cytochrome bc_1 complex is now generally accepted [1]. Evidence in support of this is the increase in the rate of photo-induced reduction of cytochrome b_h with increasing amount of ubiquinol reduced at equilibrium ($E_h < 200$ mV) or the amount of QH_2 produced under oxidizing conditions ($E_h = 220$ mV) following two (not one) successive flashes spaced at 0.7 ms [5]. It is important that in the experiments mentioned the

ubiquinol concentration was higher than that of the bc_1 complexes. The foregoing observations of the rate of cytochrome b_h reduction and $\Delta\psi$ generation provide convincing arguments that the reaction may be monomolecular when the ubiquinol concentration is no more than the concentration of bc_1 complexes. The monomolecular nature of the reaction, when $[QH_2]:[bc_1] \ll 1$, and bimolecular, when $[QH_2]:[bc_1] > 1$, may be explained by the lack of rapid quinone exchange. The ubiquinol produced in the RC is kept in a local pool and is oxidized by its 'own' (presumably neighbouring) bc_1 complex. Since the number of QH_2 molecules delivered to the pool at a time may be two, one or none (RC: bc_1 stoichiometry, 2:1), the rate of b_h reduction can change at most twice.

The accuracy of the data do not exclude the possibility of direct transfer of ubiquinol to the bc_1 complex. This may be the case, for instance, when the RC and bc_1 complex form a supercomplex under oxidizing conditions [7]. However, the experimental observations are more consistent with the concept of a local pool (see fig.2c, curves 1,2). There is, however, inconsistency with a model in which the rate of the reaction is proportional to the product of the concentrations of QH_2 and bc_1 complexes (fig.2c, curve 3). In terms of our model, the bimolecular pattern of reaction between the ubiquinol available at equilibrium and the bc_1 complex [3–6] may be explained by a change in the population of reduced ubiquinol in the local pool.

The data obtained cannot be explained in terms of the general point of view, according to which quinones form a pool common to all the RCs and bc_1 complexes. They are in keeping with the presented concept of local pools between which the exchange occurs more slowly than the reduction of cytochrome b_h .

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