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A KINETIC COMPLETION OF THE CYCLIC PHOTOSYNTHETIC ELECTRON PATHWAY OF *RHODOPSEUDOMONAS SPHAEROIDES*: CYTOCHROME *b*-CYTOCHROME c_2 OXIDATION-REDUCTION

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Summary

In *Rhodopseudomonas sphaeroides*, following a single-turnover flash of light, cytochrome c_2 is oxidized by reaction center bacteriochlorophyll, and a cytochrome *b* is reduced by the primary electron acceptor, probably via ubiquinone. In this report we show that, in the uncoupled state, the rate of re-oxidation of the cytochrome *b* is identical to the rate of reduction of the cytochrome c_2, a kinetic completion of the cyclic photosynthetic electron transport system.

While it has been recognized for many years that photosynthetic electron flow in the photosynthetic bacteria is cyclic, it has only been in the last few years that some of the individual steps of the cycle have been identified and kinetically resolved [1,2,3]. In *Rps. sphaeroides* the primary light activated charge separation occurs in the photochemical reaction center protein, where the primary donor (a bacteriochlorophyll complex, designated P) becomes oxidized, and the primary acceptor [possibly an iron-ubiquinone complex, designated photoredoxin (Pd)] becomes reduced in less than 1 ns [3]. The reduced primary acceptor then reduces the secondary acceptor, which is now generally considered to be ubiquinone [4, 5]. In *Rps. sphaeroides* this is ubiquinone-10 [6] and there are 10—20 per reaction center protein [7]. The reduction of ubiquinone in the chromatophore membrane involves the uptake of a proton from the aqueous phase, the half-time of this protonation being 150 μs at pH 7 [5,8]. The reduced ubiquinone then reduces a *b*-type cytochrome with a reduced α-band prominent at 560 nm and an oxidation-reduction midpoint potential of 50 mV at pH 7 [9]; it has been operationally designated cytochrome b_{50} [9]. The ubiquinone-cytochrome *b* reaction has a half-time of 1—2 ms, a value obtained in the presence of antimycin to inhibit the re-oxidation of the cytochrome.

During the period that the electron is moving from the electronegative primary acceptor through one or more ubiquinones to cytochrome b_{50}, the electropositive "hole" left in the oxidized reaction center bacteriochlorophyll complex (P^+) is filled by an electron from ferro-cytochrome c_2 [9]. This reaction is biphasic [10], the half-times of the oxidation phases (each about 50%) being of the order of 20 μs and 300 μs (the latter time is variable [10]). These reasonably well characterized electron transfer reactions are represented schematically in Fig. 1. Notably absent from the scheme are any kinetic details of the re-oxidation of cytochrome b_{50} and the re-reduction of cytochrome c_2 following flash-induced reduction and oxidation respectively. In this report we examine these kinetics in the energetically uncoupled state, activated by single turnover pulses.

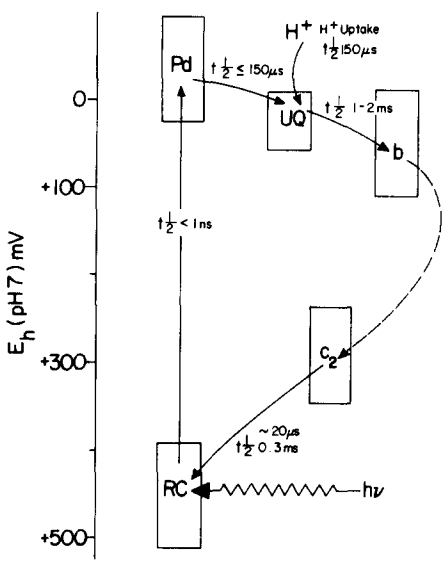

Fig.1. The cyclic electron transport pathway of *Rps. sphaeroides*.

Chromatophores were prepared from *Rps. sphaeroides* Ga cells using a French pressure cell as previously described [10]. The pulsed reactions of cytochromes *b* and *c* were monitored in the rapidly responding, unchopped dual wavelength spectrophotometer, and averaged using the computer of average transients, also as previously described [10]. The rationale of the experiments was as follows: *Rps. sphaeroides* possesses three *b*-type cytochromes [9], two of which (b_{50} and b_{155}) can be involved in flash-induced electron flow [9]. However, although b_{155} is oxidized by ferricytochrome c_2 following a single turnover flash ($t_{1/2}$ = 2 ms), its re-reduction takes several seconds. Furthermore, the oxidation and reduction reactions of cytochrome b_{155} are not sensitive to uncoupling agents, although antimycin inhibits its oxidation [9]. Thus cytochrome b_{155} appears not to be part of the cycle itself, nor is it significantly involved in electron flow induced by repetitive flashes of light within the seconds time scale. It seems clear, therefore, that under rapidly repetitive

pulsed conditions after the first flash, cytochrome b_{50} is the only *b*-type cytochrome involved in the cyclic electron flow system.

Looking at the system from the standpoint of cytochrome c_2, following the first flash it receives an electron from b_{155} but, after subsequent flashes, it receives electrons only via the cyclic chain through ubiquinone and b_{50}. Fig. 2A shows the kinetic responses of cytochromes *b* and c_2 when subjected to a train of pulses of light, each long enough (full width at half height, 6 μs) to elicit what is essentially a single turnover of the photochemical reaction center. The flashes in the train are separated by a 40 ms dark period, the length of which is chosen to permit the cytochrome b_{50} and c_2 reactions to run to completion. The changes are the average of a number of such pulses.

In Fig. 2A it can be seen that cytochrome b_{50} (monitored at 560—540 nm) undergoes a transient reduction after the flash, followed by re-oxidation which is complete by 12 ms. At the same time, cytochrome c_2 is rapidly oxidized by the flash and is then subsequently re-reduced; this is also complete within 12 ms. The addition of antimycin has a marked effect on these kinetics. This antibiotic greatly inhibits the rate of oxidation of cytochrome *b*, and in its presence it can be seen (Fig. 2B) that the extent of single-turnover flash-

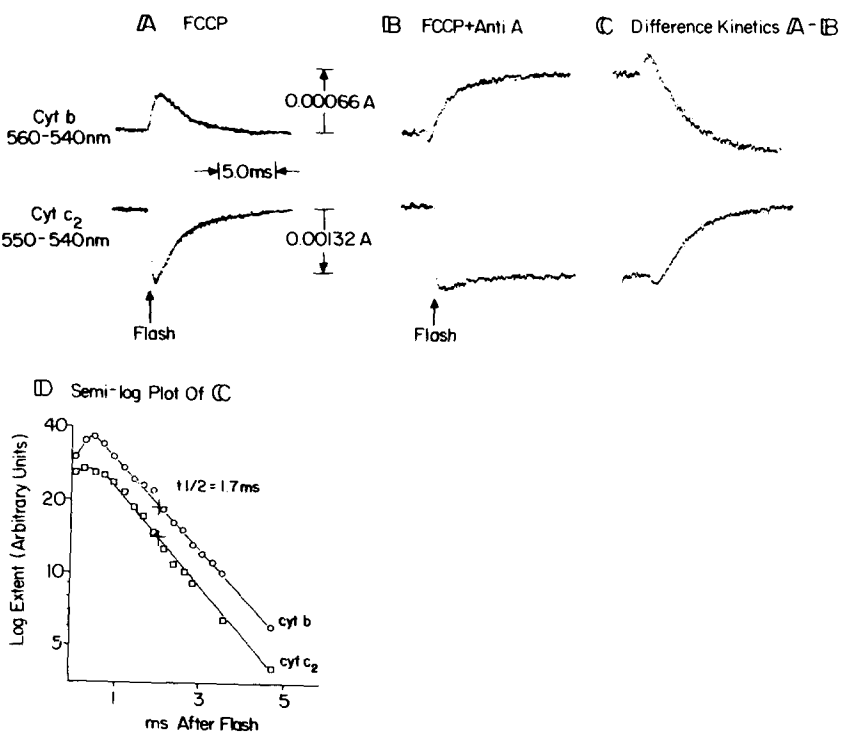

Fig. 2. The kinetics of cytochromes *b* and c_2 in *Rps. sphaeroides*. The anaerobic cuvette contained 7 ml 20 mM morpholinopropane sulfonate, 100 mM KCl, 1 mM $MgCl_2$ pH 7.0, chromatophores (20.4 μM bacteriochlorophyll), 5 μM phenazine methosulfate, phenazine ethosulfate and Diaminodurol plus 10 μM carbonylcyanide *p*-trifluoromethoxy phenylhydrazone. The traces in A and B are the average of 128 for cytochrome *b* and 64 for cytochrome c_2, the flashes being 40 ms apart in A. Antimycin was added to 2 μM in B, and under these conditions when the re-oxidation of cytochrome *b* and re-reduction of cytochrome c_2 were slow, flashes were delivered every 2 min to allow the system to relax completely between turnovers. E_h = +95 mV.

induced cytochrome *b* reduction is appropriately larger than in the absence of antimycin (Fig. 2A). The half-time of the total reduction of cytochrome *b*, revealed in the presence of antimycin, is approximately 1 ms, in agreement with a previous determination [9]. The apparent enhancement elicited by antimycin results from the inhibition of the re-oxidation of cytochrome *b* which is intrinsically competitive with the reduction in the absence of antibiotic. A similar effect is not as pronounced in the case of cytochrome c_2 (Fig. 2B) since its re-reduction rate is sufficiently slow that both reactions can be resolved in the uninhibited state.

In order to resolve the exact kinetics of the cytochrome *b* oxidation and cytochrome c_2 reduction in the absence of antimycin, Figure 2C shows the difference between two traces recorded in the presence and absence of antimycin A for cytochrome *b* and cytochrome c_2. The resolved kinetics of these two traces are almost identical (Fig. 2D); that is, the kinetics of the re-oxidation of cytochrome *b* are the same as those of the re-reduction of cytochrome c_2. Fig. 3 shows spectra of the changes, indicating that the observed kinetics do correspond to cytochrome c_2 and cytochrome *b*. Qualitatively similar results have been obtained with chromatophores from *Rps. capsulata* Ala pho$^+$.

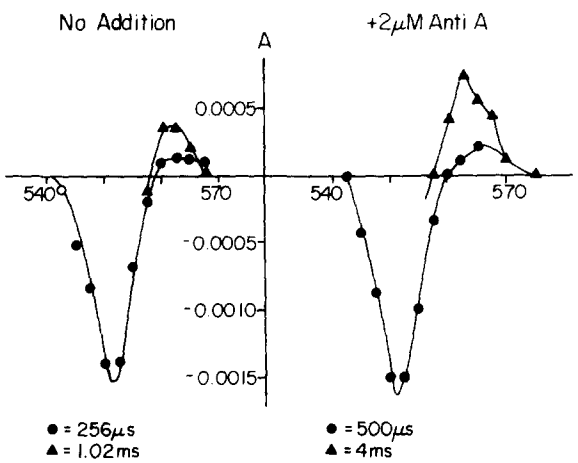

Fig.3. Spectra of the changes resolved in Fig. 2. The conditions were those of Fig. 2, except that the measuring wavelength was altered as indicated while the reference wavelength was maintained at 540 nm. Kinetic traces were obtained exactly as in Fig. 2, and the points represent the changes measured at the time indicated. E_h = 100 ± 10 mV.

Thus, in the uncoupled, free-running state, there is no kinetic impediment to electron flow from cytochrome b_{50} to cytochrome c_2. This does not necessarily rule out the possibility of other components between the two cytochromes, but we can now define at least two conditions which they must fulfill; (a) they must have intrinsically very rapid rates of reduction and oxidation, at least under these uncoupled conditions, so that these are not rate-limiting reactions in electron flow between cytochrome b_{50} and cytochrome c_2 and (b) antimycin must act on the intermediary components such that both the oxidation of cytochrome *b* and the reduction of cytochrome *c* are inhibited at once, since the kinetics of Fig. 2B are seen immediately

after the addition of antimycin, following a single turnover.

The only candidate known to date with the appropriate thermodynamic properties to act between cytochrome b_{50} and cytochrome c_2 is the "Rieske" iron-sulfur center (characterized by an EPR band of the reduced form at g 1.90, E_{m7} = +285 mV [11]). We have recently shown that this component can be photo-oxidized on a seconds time scale, but its relevancy on an ms time scale may be questioned.

In conclusion, we have identified one, perhaps extreme, kinetic completion of the cyclic electron transport system of *Rps. sphaeroides* and *Rps. capsulata.* The study of the system in the coupled state, when useful work is being done, may well reveal a more complicated situation,

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