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UBIQUINONE REDUCTION AND PROTON UPTAKE BY CHROMATOPHORES OF *RHODOPSEUDOMONAS SPHAEROIDES* R-26

PERIODICITY OF TWO IN CONSECUTIVE LIGHT FLASHES

YORAM BAROUCH and RODERICK K. CLAYTON

Division of Biological Sciences, Cornell University, Ithaca, N.Y. 14853 (U.S.A.)

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Summary

Chromatophores of *Rhodopseudomonas sphaeroides* strain R-26 were subjected to a series of brief flashes of light in the presence of diaminodurene as an electron donor. Odd-numbered flashes induced the reduction of ubiquinone to the anionic semiquinone, as indicated by absorbance changes near 450 nm. This reaction was not attended by proton binding. Even-numbered flashes caused disappearance of the semiquinone, presumably by conversion to the fully reduced form. This reaction was attended by proton uptake.

It was reported recently that in purified reaction centers of *Rhodopseudomonas sphaeroides* supplemented with ubiquinone, odd-numbered flashes of light result in the formation of ubisemiquinone, whereas fully reduced ubiquinone is formed on even-numbered flashes [1, 2]. Similar conclusions have been drawn for Photosystem II of green plants [3, 4], where plastoquinone plays a role similar to that of ubiquinone in *R. sphaeroides*. Heretofore, the oscillatory pattern of ubiquinone reduction, as observed by absorbance changes at 450 nm, has not been reported in bacterial chromatophores or in whole cells. We now report such quinone oscillations in chromatophores of the carotenoidless mutant R-26 of *R. sphaeroides*.

Proton uptake following single flashes was described in chromatophores of *Chromatium vinosum* by Chance et al. [5]. Detailed studies by Cogdell

Abbreviations: DAD, 2,3,5,6-tetramethyl-*p*-phenylenediamine; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

et al. [6, 7] and by Petty and Dutton [8], revealed the same pattern for chromatophores of the Ga (green) mutant of *R. sphaeroides*; H^+ binding after every flash. On the other hand, isolated reaction centers of strain R-26 show H^+ binding only after even-numbered flashes [2]. Here we report that in chromatophores of *R. sphaeroides* R-26, even-numbered flashes cause H^+ uptake (exhibited by a net change in the pH of the external medium) but odd-numbered flashes do not.

Purified chromatophores of *R. sphaeroides*, strains R-26 and Ga, were prepared as described before [9]. For studies of proton uptake the chromatophores were resuspended in 75 mM KCl, pH 7.2. All light-induced absorbance changes were measured with a split beam spectrophotometer [10]. Saturating actinic flashes, spaced 1 to 5 s apart, were provided by a xenon lamp as described elsewhere [1]. A Tracor-Northern TN-1500 Signal Averager was used to average more than one measurement. The concentration of reaction centers was estimated from the maximum reversible light-induced absorbance change that could be elicited at 870 nm [11]. All measurements were made with chromatophores that had been dark-adapted at 3°C for at least 6 h.

Fig. 1 shows absorbance changes at 450 nm in the presence of an electron donor that transfers electrons to the photo-produced oxidized bacteriochlorophyll. Note the oscillating pattern that suggests formation and disappearance of ubisemiquinone on consecutive flashes. Best results were obtained with 2,3,5,6-tetramethyl-*p*-phenylenediamine (DAD) as donor, but similar observations were made with *N*,*N*,*N'*,*N'*-tetramethyl-*p*-phenylenediamine (TMPD) or ascorbate, provided the exciting flashes were not more than 1—2 s apart. If these absorbance changes were due to oxidation of the added electron donor, we would expect the largest signal at 480 nm when using DAD, and no signal in the visible range of the spectrum when using ascorbate. In fact, absorbance changes at 438—475 nm were qualitatively similar to those at 450 nm, but signals were maximal near 450 nm and declined almost to zero at 480 nm. This is in agreement with spectra of ubisemiquinone and fully reduced quinone measured in isolated reaction centers [1, 2, 12].

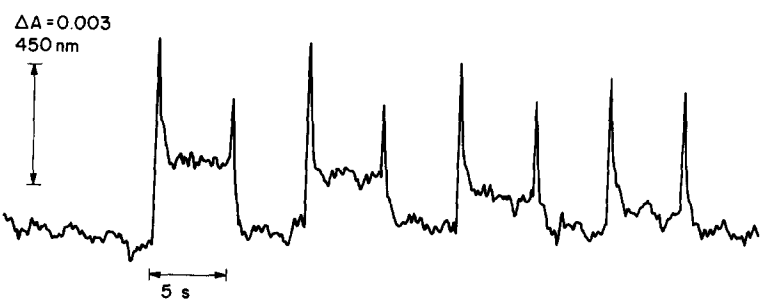

Fig. 1. Flash-induced absorbance changes at 450 nm in chromatophores of *R. sphaeroides* strain R-26. The chromatophores were suspended in 0.01 M Tris·HCl, pH 7.5, to an absorbance of 0.85 at 450 nm Diaminodurene 100 μM, was added as an electron donor. The xenon flashes, 0.1 ms duration, were presented every 5 s and passed through a Corning 7-69 (broad band near infrared) filter. The transient increases of absorbance, due to oxidation and rapid re-reduction of reaction center bacteriochlorophyll, serve to show when the flashes were presented.

Applying an approximate extinction coefficient of 2.5—3.5 mM^{-1}. cm^{-1} at 450 nm for ubisemiquinone anion in reaction centers [12, 13] we found that approximately one ubiquinone per reaction center was reduced to ubisemiquinone following odd-numbered flashes. As in isolated reaction centers [1, 2] and Photosystem II of green plants [3, 4], we conclude that in chromatophores of *R. sphaeroides* R-26 an odd-numbered flash induces the reduction of ubiquinone to ubisemiquinone, and the next (even) flash causes full reduction of the quinone. We do not know whether the quinone which is reduced in this manner is a member of the quinone pool or a distinct "secondary" quinone (UQ_2) which might serve as an intermediary between the primary quinone (UQ_1) and the pool.

Fig. 2 shows measurements of flash-induced proton uptake, using phenol red to indicate the pH of the external medium. Based on the impermeability of the membrane to the indicator [6, 8] we assume that those flash-induced absorbance increases at 560 nm that depend on the presence of the indicator reflect increases in external pH. Such flash-induced pH changes are probably a consequence of H^+ uptake by the chromatophore, but we cannot exclude other interactions of either DAD or the indicator with the chromatophores or with each other, which may affect the external pH. Fig. 2 shows that there were no net absorbance changes at 560 nm (no pH changes) following odd-numbered flashes, but even numbered flashes were followed by net increases in absorbance. These pairwise steps were observed whether or not antimycin A was present in the sample, provided the pH indicator was present and no additional buffer was added. The pattern persisted through at least ten flashes. Although we have not determined the stoichiometry accurately, we estimate that 1—2 protons disappeared from the external medium per reaction center following every even-numbered flash. In contrast to the observations described here, no similar data have been reported for the green mutant Ga, which has been studied in some detail [7, 8]. We confirmed the observation that in Ga, protons are removed from the external medium following single flashes. Cogdell et al. [6] implied that ubiquinone might be a proton binding entity, an idea that gained

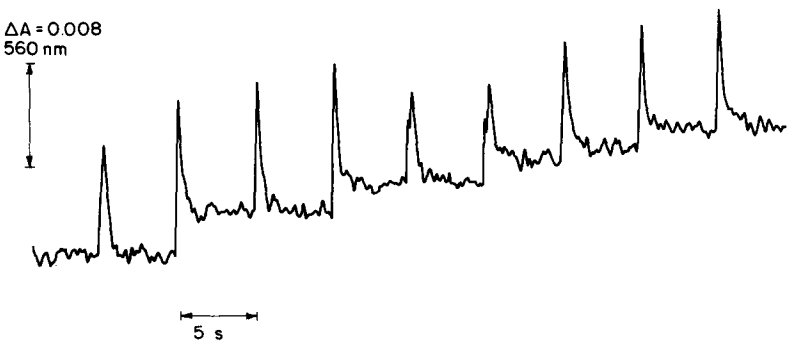

Fig. 2. Flash-induced absorbance changes at 560 nm in chromatophores of *R. sphaeroides* R-26 in the presence of 0.003 percent phenol red as pH indicator. Actinic flashes as in Fig. 1, with transient spikes due to bacteriochlorophyll oxidation showing when flashes were presented. This trace is the average of two similar traces. Chromatophores, absorbance 0.60 at 560 nm, at pH 7.2 with 75 mM KCl, 100 μM DAD, and phenol red. The stable absorbance increases following flashes 2,4,6 and 8 reflect removal of protons from the medium.

more support from further studies of Ga mentioned above. If $UQ^{-\cdot}$ is the proton binding entity in strain Ga, absorbance changes near 450 nm due to ubiquinone reduction will be difficult to detect because any changes due to formation and disappearance of $UQH^{\cdot}$ will be smaller than those due to $UQ^{-\cdot}$. Furthermore, there are much larger changes near 450 nm due to carotenoid band shifts in strain Ga. Our data suggests that in strain R-26, $UQ^{-\cdot}$ is a stable photo-product of a first flash, and no protons are taken up until a second flash induces the reaction $UQ^{-\cdot} + e^{-} \rightarrow UQ^{2-}$ (compare Figs. 1 and 2).

The difference in proton uptake between chromatophores of strains Ga and R-26 could be related to the absence of carotenoids in the latter, or to the absence of the entire "B800-B850-carotenoid" antenna pigment-protein complex [9]. We can speculate that in strain R-26, $UQ^{-\cdot}$ is not protonated either because it is masked from the external medium or because the pK of the reaction $UQ^{-\cdot} + H^{+} \rightarrow UQH^{\cdot}$ has been altered (see ref. 8). In this view the fully reduced UQ^{2-} is accessible to protonation in strain R-26. Alternatively, we can suppose that in strain Ga, the semiquinone (or its extra electron) formed at each reaction center is mobile, allowing a dismutation reaction $2UQ^{-\cdot} \rightarrow UQ^{2-} + UQ$ after each flash [14], and in strain R-26 the semiquinone formed at different reaction centers lacks sufficient mobility for such interaction. The data are compatible with this view if UQ^{2-}, but not $UQ^{-\cdot}$, can be protonated in situ.

It may be useful to study ubiquinone reduction, proton binding and quinone mobility in phenotypes of *R. sphaeroides* intermediate between Ga, which contains chloroxanthin, and R-26, which contains no polyene precursors of carotenoid pigments.

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B.G. deGrooth, J.C. Romijn and M.P.J. Pulles (Proc. 4th Int. Congr., on Photosynthesis, Reading, England, 1977) reported quinone oscillations in chromatophores of wild type *R. shpaeroides* at redox potentials above 270 mV, but not below 200 mV. They suggest that oxidation of cytochrome *b* permits oscillations by preventing electron flow from reduced cytochrome *b* to quinone. Perhaps the redox potential in our chromatophores was higher in strain R-26 than in strain Ga.