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KINETICS OF ELECTRON TRANSFER BETWEEN THE PRIMARY AND THE SECONDARY ELECTRON ACCEPTOR IN REACTION CENTERS FROM *RHODOPSEUDOMONAS SPHAEROIDES*

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Summary

Photoreduction of the two ubiquinone molecules, UQ_1 and UQ_2, bound to purified reaction center from *Rhodopseudomonas sphaeroides* induces different absorption band shifts of bacteriochlorophyll and bacteriopheophytin molecules depending on which ubiquinone is photoreduced. This allows us to study electron transfer between UQ_1 and UQ_2 directly by absorption spectrometry. The results support a model in which electrons are transferred one by one from UQ_1 to UQ_2 with a half-time of 200 μs, and two by two from fully reduced UQ_2 to the secondary acceptor pool.

Introduction

It has been shown recently that in reaction centers of *Rhodopseudomonas sphaeroides*, a periodicity of two is observed for the formation of ubisemiquinone and fully reduced ubiquinone in a series of saturating single turnover flashes [1, 2]. On each odd flash, a ubisemiquinone molecule is formed and each even flash promotes the full reduction of one molecule of ubiquinone. This oscillatory behaviour has been explained by a model in which after one charge separation, the electron is held in the reaction center until a second charge separation allows the reduction of one molecule of the ubiquinone pool in a two-electron transfer reaction. This model implies that two special ubiquinone molecules, UQ_1 and UQ_2 are coupled in a particular way to the reaction center.

Because of the lack of kinetic data, it was not possible to distinguish

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definitively between a series or a parallel mode of operation of the two ubiquinone molecules. This report describes observations on the electron transfer between the primary electron acceptor and the secondary acceptor pool. Light-induced difference spectra were measured in conditions where UQ_1 or UQ_2 is selectively photoreduced. In the visible part of the spectra, the light-induced absorbance changes are almost identical for the reduction of UQ_1 or UQ_2 and similar to the one obtained for ubisemiquinone in alcoholic solution [3]. However, the shape and amplitude of absorbance changes in the near infrared region, described as absorption band shifts of bacteriochlorophyll and bacteriopheophytin molecules, are different depending on whether UQ_1 or UQ_2 is photoreduced. These differences permit us to study directly the kinetics of electron transfer between UQ_1 and UQ_2 in the reaction center.

Reaction centers from *Rhodopseudomonas sphaeroides* were prepared as described previously [2]. Light-induced absorbance changes were measured with a conventional single beam spectrophotometer or with the split beam spectrophotometer described in Ref. 4. For flash kinetics measurement, the output of a fast amplifier (Pacific Photometric Instruments Model 62/2A44, Emeryville, Calif.) was sent to a Biomation Model 8100 digitizer (Cupertino, Calif.) coupled to a Tracor-Northern TN-1500 signal Averager. Absorption difference spectra were measured with the Cary 14R spectrophotometer as described in Ref. 5.

The optical path of the cuvette was 1 cm. Actinic illumination was provided either by a Xenon flash lamp, operated at 100 J and 0.1 ms half-time, filtered through Corning filters 4—97 (blue) or 7—69 (near infrared) or by a Q-switched ruby laser (Korad Model K-15). Adequate filters were put in front of the detector to block scattered actinic light.

Fig. 1 shows the absorbance changes at a few selected wavelengths in response to a sequence of saturating flashes, in conditions where UQ_1 and UQ_2 can be selectively photoreduced. For the traces on the right, reaction centers (2 μM) were in the presence of orthophenanthroline (2 mM), which blocks electron transfer between the primary and the secondary electron acceptor in chromatophores [6] or reaction centers [7]. Under such conditions, only the primary electron acceptor UQ_1 can be photoreduced and therefore the first flash induces an absorbance change decaying slowly in the dark. Succeeding flashes are able to promote a charge separation only in those reaction centers in which reoxidation of the primary acceptor has occurred. In the presence of ubiquinone as secondary acceptor, electron transfer is possible between reaction centers and the exogenous pool. In that case, light-induced absorbance changes exhibit an oscillating pattern of periodicity two, as has been described previously [1, 2] and as it is shown in the left-hand traces of Fig. 1. At 450 nm, which is the characteristic wavelength of the unprotonated semiubiquinone [3], the absorption increases after each odd flash corresponding to the formation of the ubiquinone UQ_2^-, and the absorption decreases after each even flash corresponding to the formation of the fully reduced ubiquinone. The semiquinone induces shifts within the absorption band of the bacteriopheophytin molecules (Fig. 1, 750 and 770 nm traces).

The absorption difference spectra linked to the photoreduction of UQ_1 and UQ_2 are plotted on Figs. 2 and 3 respectively. These difference spectra

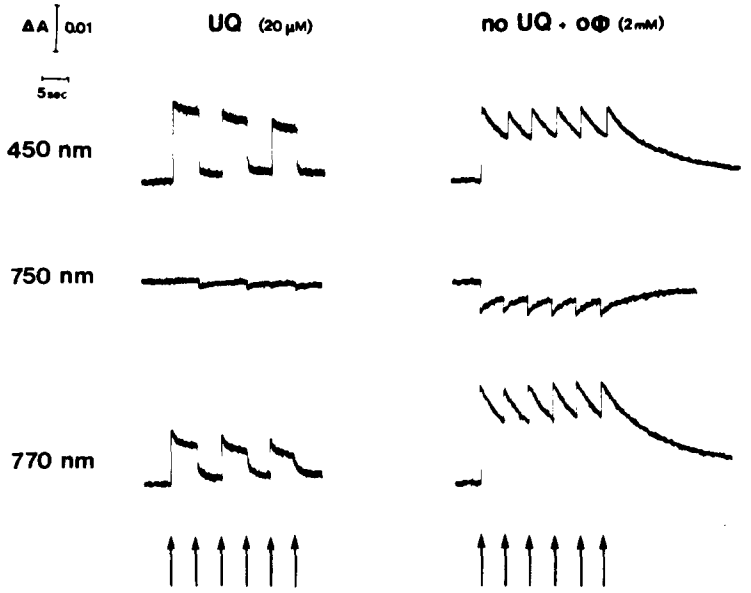

Fig. 1. Light-induced absorbance changes in reaction centers (2 μM, in 0.3% LDAO, 0.01 M Tris·HCl, pH 7.5, 100 μM diaminodurene) measured at 450, 750 and 770 nm. Actinic illumination was provided by xenon flashes spaced 5 s apart, indicated by the arrows. Left: with 20 μM exogenous ubiquinone. Right: with 2 mM *ortho*-phenanthroline and without exogenous ubiquinone.

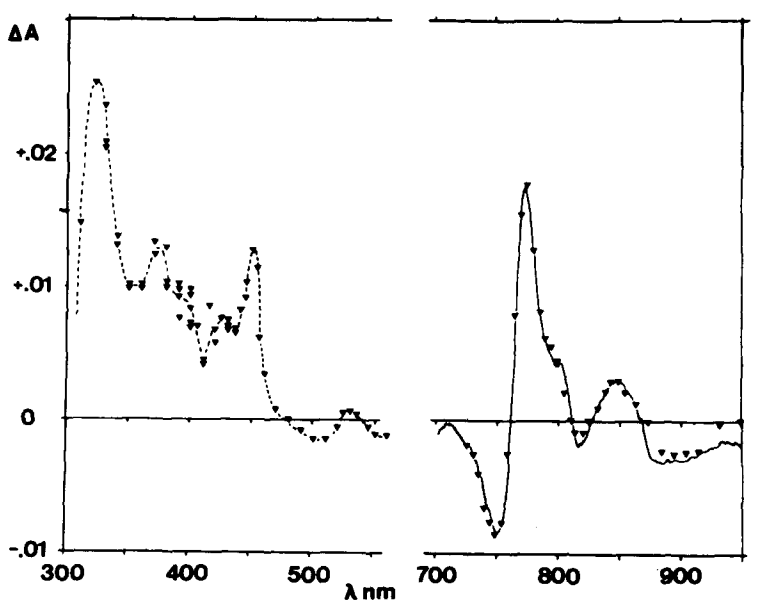

Fig. 2. Difference spectrum of absorbance changes induced by the first flash, obtained point by point from kinetic data as in Fig. 1 (right). The full line shows, for reaction centers under the same conditions, a "light-dark" difference spectrum obtained with the Cary 14R spectrophotometer. Absorption spectra were recorded in the dark and under continuous blue actinic illumination (Corning CS4-96 filter), and the difference taken.

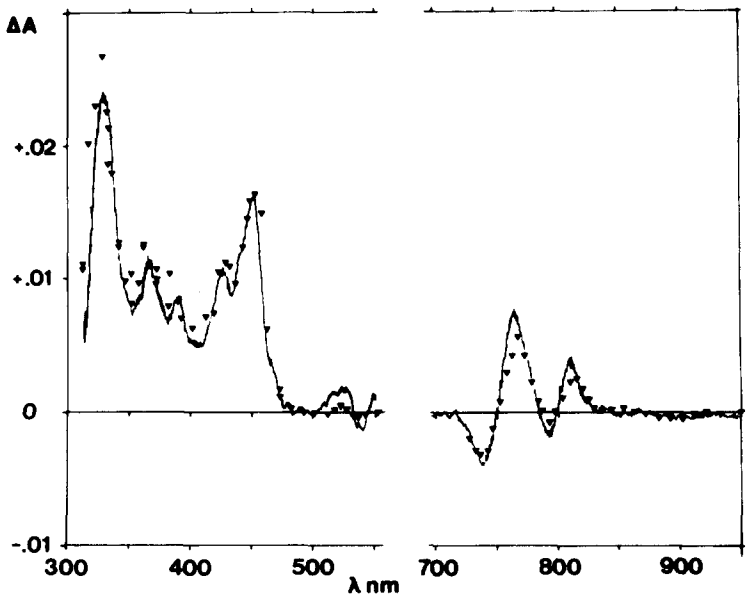

Fig. 3. Difference spectrum of absorbance charges induced by the first flash, obtained point by point from kinetic data obtained in the same conditions as for left-hand traces of Fig. 1. The full line corresponds to the difference between absorption spectra recorded in the dark with a Cary 14R spectrophotometer after one or two actinic xenon flashes (conditions as in Fig. 1, left). The time elapsed between the excitation by the flash and the end of the scan was less than 25 s.

were obtained either point by point (triangles) from kinetic data similar to those reported in Fig. 1 or with a Cary 14R spectrophotometer in one scan (full line). In order to obtain the difference spectrum induced by the reduction of UQ_2 (Fig. 3, full line), absolute absorption spectra of reaction centers were recorded in the dark after either one or two actinic flashes in the presence of UQ (20 μM) and diaminodurene (100 μM). These two spectra were stored and the second subtracted from the first. The total scan duration was less than 25 s, allowing only a small reoxidation of the ubisemiquinone formed by the actinic flash (see ref. 2). The half time for reoxidation of $UQ^{\bullet-}$ in the presence of orthophenanthroline was about 10 s under the conditions of Fig. 1 (right-hand traces). The difference (Fig. 2, full line) was therefore obtained by subtracting absolute absorption spectra of reaction centers kept in the dark or subjected to continuous illumination. This procedure could only be applied to the measurement of the infrared part of the difference spectrum, because of the accumulation of oxidized diaminodurene under continuous excitation. In both Fig. 2 and Fig. 3, there is good agreement between difference spectra recorded either point by point or by scanning. The difference spectrum induced by the reduction of UQ_1 is similar to the one obtained by Clayton and Straley [8] for the primary acceptor. A comparison with spectra recorded in alcoholic solution by Bensasson and Land [3] indicates that these reduced species are unprotonated ubisemiquinones.

Both difference spectra (Figs. 2 and 3) are almost identical for wavelengths between 300 and 500 nm. Additional changes are also observed in the near infrared region and the neighbourhood of the bacteriopheophytin Q_X

transition (approx. 535 nm). These changes can be interpreted as absorption band shifts of bacteriochlorophyll and bacteriopheophytin molecules due to electrostatic interaction with the semiquinones. The shapes and amplitudes are different in case of reduction of UQ_1 or UQ_2. For example, the reduction of UQ_1 induces a red shift of the Q_X transition of bacteriopheophytin, whereas the reduction of UQ_2 induces a blue shift. In addition, isobestic points in the region of the Q_Y transition of bacteriopheophytin occur at 755 and 750 nm for reduction of UQ_1 or UQ_2 respectively. These differences in shape and amplitude can be expected because of the importance of geometrical factors (distance and respective orientation of the reduced molecules and of pigments) in determining the electrostatic effects.

The different absorption changes in case of reduction of UQ_1 or of UQ_2, enable us to test the "series" model by studying the kinetics of the flash-induced changes at selected wavelengths. Absorption changes occurring in the millisecond time range after one or two actinic flashes are presented in Fig. 4. A few wavelengths are selected where semireduced quinone (450 nm), bacteriopheophytin band shift (750 and 770 nm) and oxidized *P*-870 (850 nm) can be observed. There is no change in the signal due to oxidized *P*-870, detected at 850 nm, during the time of detection. At 450 nm, a transient is apparent on

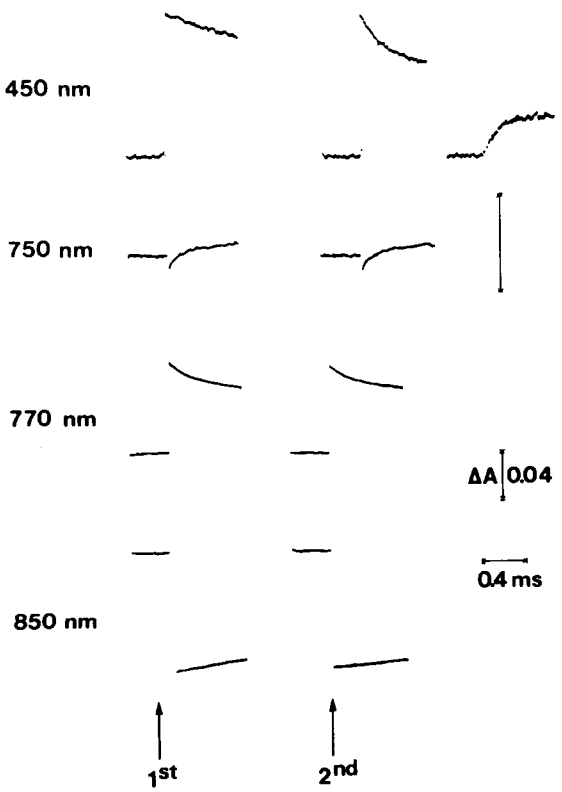

Fig. 4. Kinetics of light-induced absorbance changes at 450, 750, 770 and 850 nm, following the first and the second laser flash for reaction centers (2 μM) in the presence of ubiquinone (20 μM) and diaminodurene (100 μM). The dark interval between flashes was 10 s. The trace in the upper right shows the difference between the two traces obtained at 450 nm.

the second flash. The half time of this transient absorption change is 200 μs, as shown more clearly by the difference between the absorbance changes occurring on the first and second flash (upper trace on the right in Fig. 4). This transient phase corresponds to the dismutation of the two ubisemiquinones UQ_1^- and UQ_2^-, leading to oxidized UQ_1 and fully reduced UQ_2. At wavelengths where reduction of UQ_1 and UQ_2 induce different absorption changes, i.e., 750 and 770 nm, a transient with a half-time of 200 μs is observed on the first flash. If one plots the amplitude of the 200 μs phase occurring on the first flash versus wavelength, a difference spectrum is obtained that is similar to the difference spectrum between the absorbance changes induced by the reduction of UQ_1 and UQ_2 for wavelengths below 810 nm. In the presence of 2 mM orthophenanthroline, no 200 μs phase is observed in the absorbance changes, either on the first or successive flashes for all wavelength tested (data not shown). These results are consistent with an electron transfer between UQ_1 and UQ_2 after each actinic flash, occurring with a half-time of 200 μs.

However, the amplitude of the 200 μs phase on the second flash, for example at 770 nm, is smaller than expected. According to the spectra of Figs. 2 and 3, one would expect the fast phase to be three times as large on the second flash as on the first one. The amplitude of the transient at 770 nm after the first flash should correspond to the difference between Figs. 3 and 2. The transient after the second flash corresponds to the disappearance of UQ_1^- and UQ_2^-, and the appearance of UQ_1 and fully reduced UQ_2. Fully reduced UQ_2 does not cause an absorbance change at 770 nm (left-hand trace, Fig. 1). Therefore the transient after the second flash should have an amplitude corresponding to the negative sum of the 770 nm absorbances in Figs. 2 and 3. This anomalous point could be explained by the fact that for the difference spectra reported in Figs. 2 and 3, *P*-870 was already rereduced by the electron donor, but for the fast kinetic experiments (Fig. 4) *P*-870 was still oxidized. In other words, the positive charge on *P*-870 could affect the electrostatic effect of the negative charge on the ubisemiquinone.

Taking advantage of the different electrostatic effects induced by the reduction of the two bound ubiquinone molecules [9], we have shown that electrons are transferred one by one from the primary electron acceptor UQ_1 to a special secondary electron acceptor UQ_2. The half time of this reaction is 200 μs, and does not depend on the state of reduction of UQ_2. Electrons are then transferred two by two from this specialized secondary acceptor UQ_2 to the pool of ubiquinone. The electron transfer mechanism can be schematised as follows:

$$UQ_1\,UQ_2\,UQ_{pool} \xrightarrow{h\nu_1} UQ_1^-\,UQ_2\,UQ_{pool} \xrightarrow{t_{1/4}=200\ \mu s} UQ_1\,UQ_2^-\,UQ_{pool}$$

(stable a few min in the dark)

$$\downarrow h\nu_2$$

$$UQ_1^-\,UQ_2^-\,UQ_{pool}$$

$$\downarrow t_{1/4}=200\ \mu s$$

We do not yet know which step involves H^+ binding by the reaction center.

Acknowledgements

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