MODULATION OF THE PRIMARY ELECTRON TRANSFER RATE IN PHOTOSYNTHETIC REACTION CENTERS BY REDUCTION OF A SECONDARY ACCEPTOR

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ABSTRACT Photosynthetic application of picosecond spectroscopic techniques to bacterial reaction centers has led to a much greater understanding of the chemical nature of the initial steps of photosynthesis. Within 10 ps after excitation, a charge transfer complex is formed between the primary donor, a "special pair" of bacteriochlorophyll molecules, and a transient acceptor involving bacteriopheophytin. This complex subsequently decays in about 120 ps by donating the electron to a metastable acceptor, a tightly bound quinone.

Recent experiments with conventional optical and ESR techniques have shown that when reaction centers are illuminated by a series of single turnover flashes in the presence of excess electron donors and acceptors, a stable, anionic ubisemiquinone is formed on odd flashes and destroyed on even flashes, suggesting that the acceptor region contains a second quinone that acts as a two-electron gate between the reaction center and subsequent electron transport events involving the quinone pool.

Utilizing standard picosecond techniques, we have examined the decay of the charge transfer complex in reaction centers in the presence of the stable semiquinone, formed by flash illumination with a dye laser 10 s before excitation by a picosecond pulse. In this state the decay rate for the charge transfer complex is considerably slower than when no electron is present in the quinone acceptor region. This indicates fairly strong coupling between constituents of the reaction center-quinone acceptor complex and may provide a probe into the relative positions of the various components.

INTRODUCTION

Knowledge of the primary electron transfer events occurring during bacterial photosynthesis has been greatly enhanced through the application of picosecond absorption spectroscopy (1–4). This technique has shown that an electron is rapidly transferred (< 10 ps) from a "special pair" bacteriochlorophyll molecule (P870) to an intermediate species, I, known to involve bacteriopheophytin. Transfer to I is accompanied by a rise in absorption at 630 nm and longer wavelengths, and by a bleaching in absorption peaked at 544 nm (1, 2, 5). This intermediate then transfers an electron to a tightly bound quinone-iron complex (Q_1) with a characteristic half time of $\cong 100$ ps.

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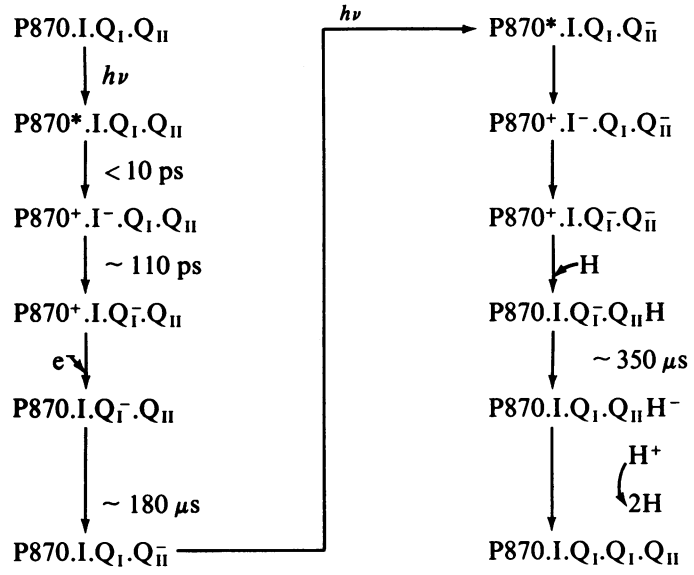
Recently, a second quinone molecule (Q_{II}) has been implicated in the transfer scheme, and a periodicity of two has been reported for a flash formation of ubisemiquinone (6, 7). Q_{II} was suggested to act as a two electron gate between Q_I and a quinone pool. Electron transfer from Q_I to Q_{II} has recently been confirmed by time-dependent optical absorption spectroscopy (8), and the following schematic combines the picosecond events with the quinone acceptor region kinetics:



This report describes changes in the rate of electron transfer between I and Q_I due to the presence of ubisemiquinone (Q_{II}^-) as measured by picosecond absorption techniques. It also provides evidence for physical changes occurring in the reaction center in response to the two-electron gating process.

MATERIALS AND METHODS

The 640-nm absorption data were obtained with a standard picosecond absorption apparatus (1, 9). It consisted of an echelon to convert time to spatial information and a double beam to compensate for fluctuations in the laser intensity. Detection was accomplished with a vidicon camera, and the output was digitized and stored in a minicomputer for further data reduction.

For absorption measurements at 544 nm, the excitation light was Raman-shifted in methylcyclohexane from 528 to ~625 nm. This shift minimized scattering effects as well as reducing the background shift due to oxidized cytochrome *c*.

Reaction centers containing ubisemiquinone (Q_{II}^-) were prepared by excitation, with a saturating flash from a dye laser (300 ns, 10 mJ, λ_{max} = 590 nm), $\cong$ 10 s before the picosecond measurement. This time was sufficient for transfer of an electron from Q_I to Q_{II} (~200 μs) but short compared to the lifetime of the semiquinone (Q_{II}^-), which is stable for many minutes (6).

Reaction centers were prepared from *Rhodopseudomonas sphaeroides*, R26, by detergent fractionation using lauryldimethylamine *N*-oxide (LDAO) as has been described previously (6, 7). Experiments utilized 200 μl of 80-μM reaction centers with 10 μl of 5 mM reduced cytochrome

c. The cytochrome *c* served to reduce $P870^+$ before the second flash. Diaminodurene (DAD), 10 μl of a 5 mM solution, was added every 60 min to insure that the cytochrome *c* remained reduced in the presence of LDAO and oxygen.

Samples were mounted in kinematic holders so that the 2-mm sample cells could be precisely replaced. This allowed alternation of the single-flash samples (i.e., those with no preflash and thus no ubisemiquinone) and the double-flash samples (ubisemiquinone present). Care was taken to insure that both single- and double-flash samples received equivalent exposure to intense light. 15 min of dark time was allowed (for each sample) between runs to obtain a consistent initial sample state.

RESULTS AND DISCUSSION

A rapid increase and subsequent decay of absorption at 640 nm has been observed by two groups (1, 2). This kinetic behavior has been attributed to the decay of the bacteriopheophytin anion as it donates an electron to the primary acceptor (Q_1). Fig. 1 shows this decay for reaction centers prepared with ubisemiquinone (double-flash) and without ubisemiquinone (single-flash). Similar results were obtained with three different reaction center preparations. Each curve is a compilation of at least 24 separate measurements. The average error bar for each point is ± 0.018.

Application of a nonlinear curve fitting routine (11) to these data shows that the half time for electron transfer in the presence of ubisemiquinone is distinctly slower than

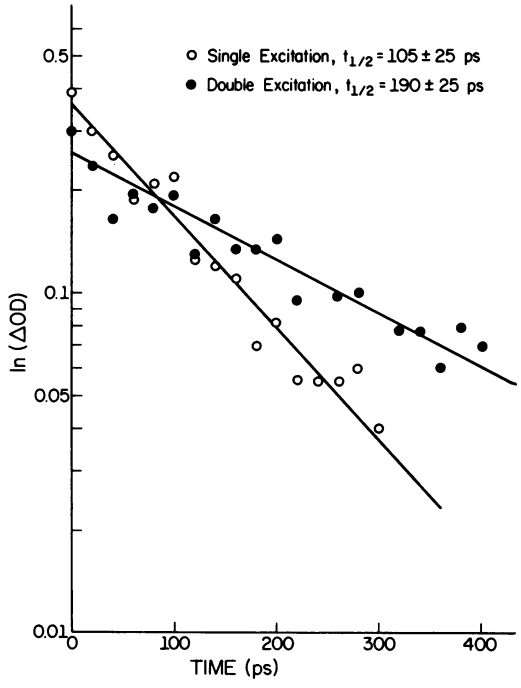


FIGURE 1 A comparison of the decay rate of absorption at λ = 640 nm for reaction centers in the presence of ubisemiquinone (●) and in the absence of ubisemiquinone (○). Each point has a standard deviation unit of 0.018.

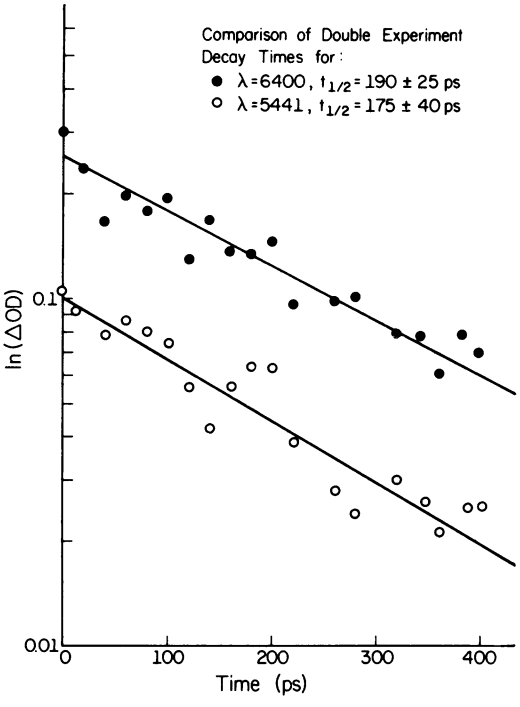


FIGURE 2

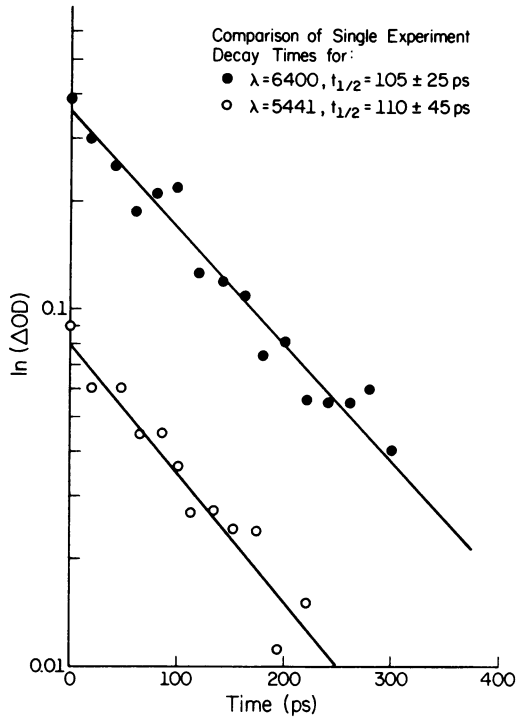


FIGURE 3

FIGURE 2 A comparison of the decay of absorption at λ = 640 nm (●) with the decay of bleaching at λ = 544 nm (○) for reaction centers in the presence of ubisemiquinone. Standard deviations ± 0.018 at 640 nm and ± 0.035 at 544 nm.

FIGURE 3 A comparison of the decay of absorption at λ = 640 nm (●) with the decay of bleaching at λ = 544 nm (○) for reaction centers in the absence of ubisemiquinone. See Fig. 2 for standard deviations.

with no ubisemiquinone present. Measurements at 700 ps (data not shown) are in close agreement with the decay measured both in the presence and in the absence of ubisemiquinone. For these samples only a slight negative (0.005) OD change was observed for single flash (no ubisemiquinone) experiments. For samples prepared with ubisemiquinone (double-flash), a residual positive OD change (0.05) was observed.

There is some disagreement in the literature on the precise value of the transfer time from I^- to Q_I (1, 2). We have consistently observed a value for the reaction half time of 105 ± 25 ps for single-flash samples, in agreement with results obtained at the Bell Telephone Laboratories (1). Our new results show that this transfer time is sensitive to local perturbations, and the discrepancies in this measurement may reflect the state or integrity of the preparation.

Bleaching at 544 nm and absorption at 640 nm are both taken to indicate I^- (1, 2). Figs. 2 and 3 compare kinetic measurements at 544 and 640 nm in the presence and absence of ubisemiquinone, respectively; the two wavelengths show similar effects. How-

ever, the optical density changes at 544 nm are somewhat smaller, and there is a large, variable contribution to the absorbance arising from the photooxidized cytochrome. The standard deviation is thus larger (±0.035), the signal-to-noise ratio smaller, and the difference between the single- and double-pulse kinetics less dramatic.

The occurrence of certain photosynthetic electron transfer events at very low temperatures has led to the application of established theoretical descriptions of electron transfer to these processes (12–17). For example, electron transfer in photosynthetic systems has been extensively studied between cytochrome *c* and the bacteriochlorophyll dimer in the purple sulfur bacterium, *Chromatium vinosum* (12), and several mechanisms have been proposed to explain the temperature dependence of the observed electron transfer rate. Although the importance of Franck-Condon factors in controlling electron transfer processes has been known for some time, only recently has it been applied to biological systems (13–17). A vibronically assisted tunneling mechanism, which incorporates the Franck-Condon factors in a manner analogous to the Förster-Dexter theory for energy transfer, has been used by Hopfield to account for both the temperature dependence of the transfer reaction in *Chromatium* and the rates for the forward and backward electron transfer reactions in *R. sphaeroides* (14, 17). Jortner (16) has successfully applied nonadiabactic multiphonon theory to the temperature dependence of the electron transfer in *Chromatium*. Even though these approaches differ somewhat in their model for the electron transfer mechanism, they do contain the effect of Franck-Condon overlap on the transition rate. Differences in the two theories lead to disagreement at low temperatures, but at high temperatures both theories correspond to the Marcus theory for electron transfer (18). At high temperatures, the transfer rate is thus given by $K = \eta e^{-(\Delta U/RT)}$, where η is the probability for transition of the electron from the potential surface of the donor to the acceptor. For nonadiabatic reactions η must be small. ΔU is the height of the crossing point for the two curves above the equilibrium position of the donor and is therefore an energy of activation for the reaction. The rate of electron transfer can be modulated by a change in either η or ΔU. The transition probability, η, is highly sensitive to configurational changes in the reaction center but would remain constant if the energies of the donor and acceptor potential surfaces change relative to one another. The apparent activation energy, ΔU, can be modified either by configurational changes in the protein or by changes in the relative energies of the two potential surfaces.

If the modification of the electron transfer rate is the result solely of an increase in ΔU, one calculates a change in ΔU of 1.3×10^3 $Jmol^{-1}$ (0.014 eV). A possible source of this increase is the electrostatic potential surface arising from the anionic semiquinone (Q_{II}^-). Assuming a colinear geometry for BPh, Q_I, and Q_{II}, a separation of 20 Å from the center of Q_{II} to the edge (near BPh) of Q_I, 3 Å as the edge-to-edge distance between BPh and Q_I, and a dielectric strength of three for the protein, a change in ΔU of 2.8×10^3 $J \cdot mol^{-1}$ (0.029 eV) can be calculated. In view of the arbitrary choice of geometry and distance parameters, this qualitative agreement indicates the feasibility of this interpretation.

A change in the barrier height need not arise solely from electrostatic interactions

but could also result from a small increase in the distance between the donor and acceptor or modifications in the protein structure. Changes in protein configuration could also account for a decrease in the energy of I^-. Recent work shows that there is pH-dependent behavior within the acceptor-quinone complex giving rise to charge alterations within it,[1] which could also lead to modulation of the picosecond transfer rate. This pH dependence is currently under investigation.

CONCLUSION

Interaction of the secondary quinone acceptor with the reaction center unit is much stronger than might have been anticipated. Despite the fact that this ubiquinone is rather weakly bound to the reaction center, the reduction of Q_{II} to ubisemiquinone can induce a retardation in the rate of electron transfer between I and Q_I. This effect could be accounted for by the electrostatic influence of the anionic ubisemiquinone (Q_{II}^-) or by a configurational change in response to this species, causing a small extra separation between I and Q_I.

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DISCUSSION

RENTZEPIS: To begin with an anonymous question: In your paper the use of nonlinear curve fitting obscures the basic question of how the base-line treatment affects the kinetic conclusion. What was the prepulse base line to the long-term base line used for Fig. 1?

KAUFMANN: The optical density changes in a sample as a result of excitation are measured with a dual-beam spectrometer. Each laser pulse is split into two beams, one passing through the air and one passing through the sample. The intensity of these two are recorded simultaneously, allowing a relative optical density (OD) to be derived. The optical density change (ΔOD) between excited and normal samples is measured by taking two successive laser shots. The first shot is taken in the absence of excitation and the second when the sample is excited. The ΔOD is, then, the ratio of the relative ODs found for these two shots.

The ratio of ODs used to determine the ΔOD of samples not prepulsed was developed using two shots, one without and one with excitation. The samples were dark-adapted for 15 min before each shot. The ΔOD for prepulsed samples was also calculated from the results of two shots, one without and one with excitation. However, after dark adaptation, the sample was illuminated with a dye laser pulse 10 s before measurements.

In this manner the ΔODs of samples in the presence and absence of a prepulse could be compared without interference from cytochrome and DAD changes arising from the prepulse. In spite of this care there was a difference in the residual ΔOD changes seen for the prepulsed and the nonprepulsed samples at 700 ps. In each case the OD measured at 700 ps was chosen as the base line for the calculation of lifetimes. One explanation for this relatively constant background is that it represents differences in the absorption spectrum of reaction centers containing $Q_I - Q_{II}$ and those containing $Q_I^- Q_{II}^-$.

The use of a nonlinear curve-fitting routine is standard in calculating exponential lifetimes. It favors points with the largest signal-to-noise ratio, providing a more accurate lifetime than a linear least squares analysis. In any case a linear least squares fit gives essentially the same lifetime as the nonlinear treatment, but the confidence limits are larger.

RENTZEPIS: Second question. What has the Arrhenius equation in your results section ($K = \eta e^{-(\Delta v/RT)}$) to do with the discussion preceding it?