PICOSECOND KINETICS IN REACTION CENTERS OF RPS. SPHAEROIDES AND THE EFFECTS OF UBIQUINONE EXTRACTION AND RECONSTITUTION

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Following picosecond light activation, the bacteriochlorophyll and bacteriopheophytin complement of Rps. sphaeroides reaction centers depleted of ubiquinone behaves as though it has no primary electron acceptor; the excited intermediary BChl/BPh† state formed in <10 ps lasts >1 ns. Addition of ubiquinone-10 reconstitutes the very rapid electron transfer rates from the excited intermediary BChl/BPh state to ubiquinone; the kinetics and rate are similar to that encountered in the untreated reaction centers. Interpretation of the data presented suggests that ubiquinone is the immediate electron acceptor from BPh^{-}. This is consistent with the model for the primary reactions leading to [$(BChl)_2^{+}$ BPh]Q^{-}.

INTRODUCTION

It is generally accepted that the primary electron acceptor of Rhodopseudomonas sphaeroides reaction centers is ubiquinone-10 associated in some way with iron (1-3). Ubiquinone (4,5) and not the iron (see 5) is essential for normal photochemistry (5) which leads to the stable formation (milliseconds) of the oxidized reaction center bacteriochlorophyll dimer $(BChl)_2^{+}$ of P870. Studies in the picosecond time range have suggested that before the reduction of the primary acceptor, an intermediary state in the bacteriochlorophyll (BChl) and bacteriopheophytin (BPh) complement (6,7) is formed in <10 ps. This state is composed of the oxidized BChl dimer $(BChl)_2^{+}$ and a reduced component [designated I^{-} (8)]† which is not the

† BChl and BPh refer to bacteriochlorophyll and bacteriopheophytin, respectively.

I designates an intermediate reduced state or species other than the primary acceptor (8).

primary acceptor (8). Fajer et al (9) have shown that I^- has spectrophotometric characteristics of BPh^- which suggests the intermediary state is $[(BChl)_2^+ BPh^-]$. The decay of the intermediary state (i.e., oxidation of BPh^-) in 100-200 ps is interpreted as indicating the rate of reduction of the ubiquinone (iron) primary acceptor (7, 10). With the primary acceptor chemically reduced so that the 100-200 ps reaction is blocked, the light activated $[(BChl)_2^+ BPh^-]$ state lasts longer, having a decay half-time of about 10 ns (11, 12).

Recently, effective methods (4, 5) for the extraction and reconstitution of ubiquinone from reaction centers have shown that removal of ubiquinone eliminates detection of the light induced $(BChl)_2^+$ in the microsecond time domain. Re-addition of ubiquinone reconstitutes light induced $(BChl)_2^+$ formation at ambient (4, 5) or liquid helium (5) temperatures. The procedures used in these analyses would not detect short lived states of the BChl/BPh complement. In this report we describe picosecond studies to determine the kinetic behavior of the intermediary state, which for simplicity we tentatively designate $[(BChl)_2^+ BPh^-]$, in ubiquinone depleted and reconstituted reaction centers.

MATERIALS AND METHODS

Reaction centers from Rps. sphaeroides strain R26 were prepared after the method of Clayton and Wang (13). Ubiquinone-10 was extracted and subsequently re-incorporated as described by Okamura et al (5). For reconstitution, ubiquinone-10 (Sigma Chemical Co.) was added in a ten fold molar excess to 50 μM extracted reaction centers in 10 mM Tris HCl 0.1% lauryldimethylamine-N-oxide pH 8.0. Excess ubiquinone was removed after 10 hours by two ammonium sulfate precipitations of the reaction centers.

Picosecond spectrometry was as described by Rentzepis et al (14, 15). 530 nm excitation came from a frequency doubled single pulse of a mode-locked Nd/glass laser; 626 nm light was obtained from Raman shifting the 530 nm light in cyclohexane (10 cm light path). Millisecond kinetics were analyzed as described previously by Dutton et al (16) using a Q-switched ruby (694 nm) laser.

RESULTS

Figure 1A shows the laser flash induced kinetics of reaction center $(BChl)_2$ oxidation measured at 605 nm on a millisecond time scale. In

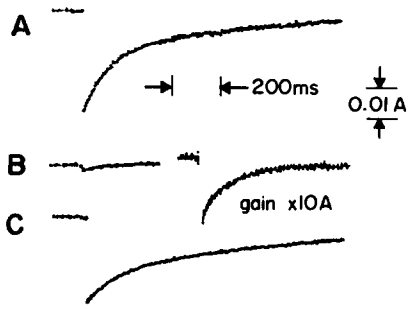

Figure 1: Reaction center $(BChl)_2$ oxidation-reduction in untreated (A) ubiquinone extracted (B) and ubiquinone-10 reconstituted (C) reaction centers: 1 μM; 1 cm light path at 605 nm-540 nm. Absorbance decrease is a downward movement. In B the trace on the right is amplified ten times in absorbance change.

untreated reaction centers the oxidation of the $(BChl)_2$ is immediate and re-reduction occurs as the electron comes back from the primary [and secondary quinone (see refs. 4, 5)] acceptors in the tens of milliseconds. In reaction centers depleted of ubiquinone (figure 1B) the extent of oxidation is only 5% of that seen in unextracted reaction centers, consistent with the extraction of 95% of the functionally essential primary ubiquinone. In ubiquinone reconstituted reaction centers (figure 1C) normal BChl oxidation is once again observable for many milliseconds. These data are consistent with previous findings (4, 5).

Figure 2 shows companion studies done in the picosecond time range, measuring what is believed to be the $BPh^{\cdot -}$ of the proposed intermediary $[(BChl)_2^{+\cdot}\ BPh^{\cdot -}]$ state at 640 nm activating with 530 nm light, or at 540 nm activating with 626 nm light. Figure 2A shows the normal formation of the intermediary $[(BChl)_2^{+\cdot}\ BPh^{\cdot -}]$ state within 10 ps (8) and its subsequent 100-200 ps oxidation half-time, leading to the long-lived $[(BChl)_2^{+\cdot}\ BPh]Q^{\cdot -}$ state as seen in figure 1. Figure 2B shows that in ubiquinone depleted reaction centers the intermediary state does not decay significantly over the 600 ps experimental time-range, consistent with ubiquinone being the immediate electron acceptor from $BPh^{\cdot -}$ (the decay half-time under these

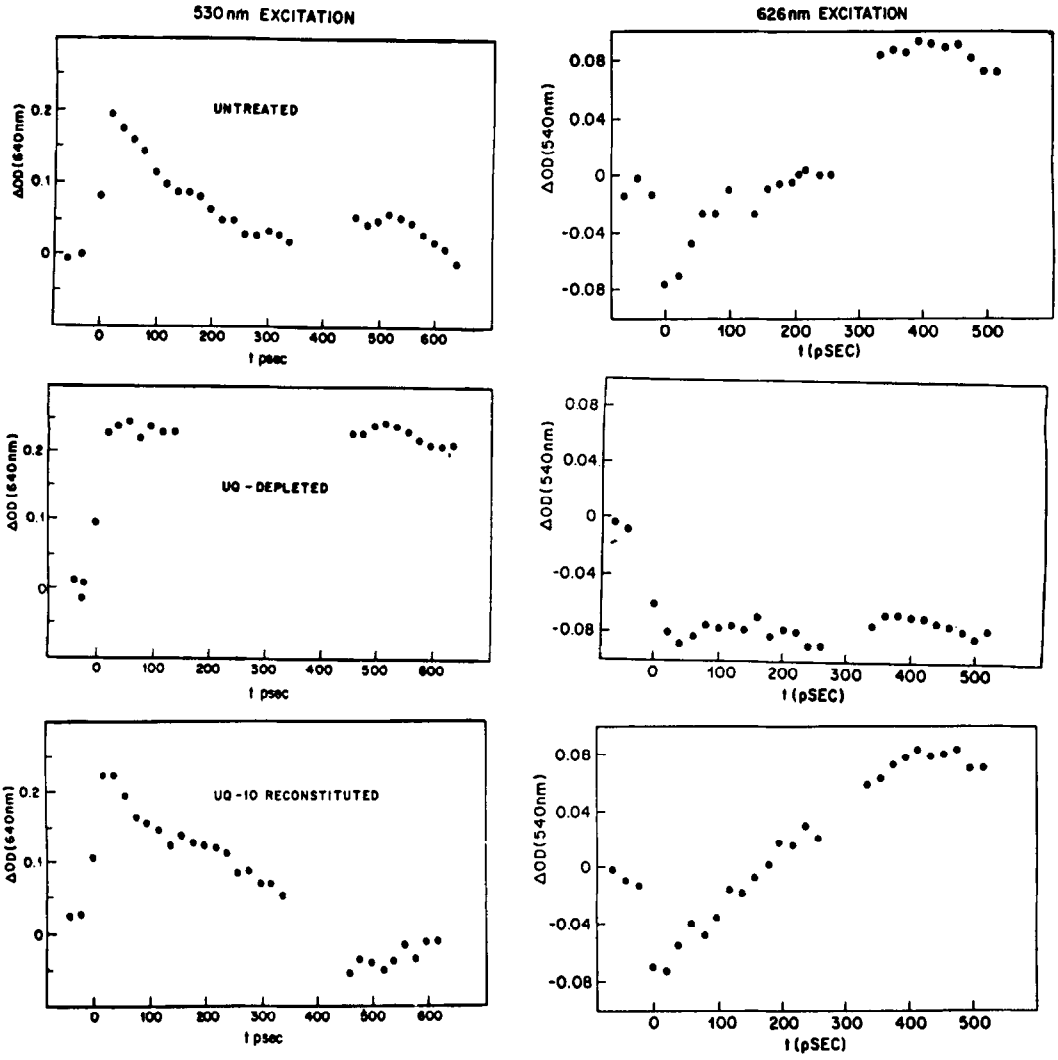

Figure 2: Reaction center BPh reduction-oxidation in untreated (A), ubiquinone extracted (B) and ubiquinone-10 reconstituted reaction centers (C): 120 μM; 1 mm light path measured in each case at 540 nm and 640 nm. Absorbance decrease is a downward movement. In A and C the absorbance increase at the end of the 540 nm traces is considered to arise from $(BChl)_2^{+\cdot}$ in the final $[(BChl)_2^{+\cdot} BPh]Q^{-\cdot}$ state.

blocked conditions might be expected to be ∿10 ns, see ref. 11). After recombination with ubiquinone, normal oxidation of $BPh^{-\cdot}$ of the intermediary state occurs in practically the same time as in the untreated reaction centers (figure 2C). This remarkable view of the reconstitution shows that the physical situation of the recombined ubiquinone in the reaction center does

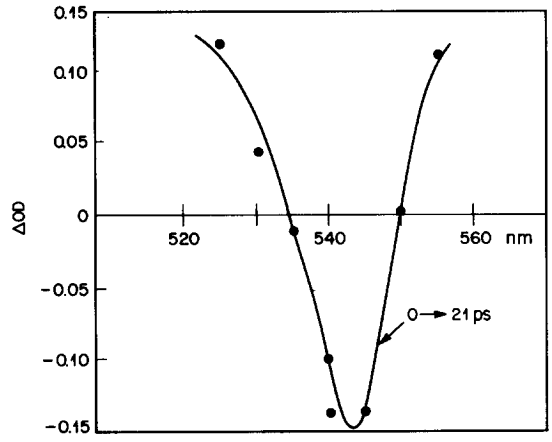

Figure 3: Spectrum measured in photoexcited reaction centers which are untreated and capable of normal forward photochemistry. Absorption change measured 21 ps after the flash in the region of the 535 nm BPh absorbance bands: 240 μM reaction centers; 1 mm light path.

not present any rate limitations which exceed those which normally govern the electron transfer from $BPh^{\bullet-}$ to the ubiquinone in the untreated reaction center.

The use of 626 nm activating light has enabled us to have a closer look at the bleaching in the 535 nm region; this was difficult before in the picosecond time range because 530 nm light was used for activation. The spectrum in figure 3 shows that the minimum is approximately 542 nm. This is the same as that generated by a nanosecond flash in reaction centers in which the primary acceptor had been chemically reduced before activation (11).

DISCUSSION

These direct measurements support the obligate role of ubiquinone for successful completion of photochemical events in the reaction center protein. The measurements suggest that ubiquinone is the immediate electron acceptor from what appears to be $BPh^{\bullet-}$; this is consistent with our working model for these early reactions leading to the $[(BChl)_2^{+\bullet} BPh]Q^{\bullet-}$ state (8). This state is stable for tens of milliseconds and functions

in vivo to oxidize cytochrome *c* and reduce secondary quinones and cytochrome *b* (16, 18-21). It is clear that ubiquinone readily goes back into the ubiquinone-depleted reaction center in a way which satisfies structural and physical demands necessary for a normal forward electron transfer from $BPh^{-\bullet}$ in the picosecond time scale. A similar conclusion regarding the physical location of ubiquinone in reconstituted reaction centers with respect to another component of the reaction center protein, $(BChl)_2$, has been made (5) based on electron tunnelling considerations for the 20 ms dark return of the electron from $Q^{-\bullet}$ to $(BChl)_2^{+\bullet}$ at liquid helium temperatures.

The 542 nm bleaching within 10 ps brings into this time domain the simple idea (17) that of the two BPh which contribute to the ~535 nm absorptions in reaction centers, it is the longer wavelength species which undergoes reduction in the proposed $[(BChl)_2^{+\bullet}\ BPh^{-\bullet}]$ state. The identification of the 542 nm BPh species in both the picosecond and nanosecond time ranges leaves the shorter wavelength species functionally undetected even on picosecond transient basis. If this is so, the functional roles of the other BPh and the two BChl moieties of P800 have still to be revealed.

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REFERENCES

1. Bolton, J.R. and Cost, K. (1973) Photochem. Photobiol. 18, 417-422.
2. Feher, G., Okamura, M.Y. and McElroy, J.D. (1972) Biochim. Biophys. Acta 267, 222-226.
3. Dutton, P.L., Leigh, J.S. and Reed, D.W. (1973) Biochim. Biophys. Acta 292, 654-664.
4. Cogdell, R.J., Brune, D.C. and Clayton, R.K. (1974) FEBS Letts. 45, 344-347.
5. Okamura, M.Y., Isaacson, R.A. and Feher, G. (1975) Proc. Nat'l. Acad. Sci. U.S.A. 72, 3491-3495.
6. Netzel, T.L., Rentzepis, P.M. and Leigh, J.S. (1973) Science 182, 238-241.
7. Kaufmann, K.J., Dutton, P.L., Netzel, T.L., Leigh, J.S. and Rentzepis, P.M. (1975) Science 188, 1301-1304.
8. Dutton, P.L., Kaufmann, K.J., Chance, B., and Rentzepis, P.M. (1975) FEBS Letts. 60, 275-280.
9. Fajer, J., Brune, D.C., Davis, M.S., Forman, A. and Spaulding, L.D. (1975) Proc. Nat'l. Acad. Sci. U.S.A. 72, 4956-4960.

10. Rockley, M.G., Windsor, M.W., Cogdell, R.J. and Parson, W.W. (1975) Proc. Nat'l. Acad. Sci. U.S.A. 72, 2251-2255.
11. Parson, W.W., Clayton, R.K. and Cogdell, R.J. (1975) Biochim. Biophys. Acta 387, 265-278.
12. Cogdell, R.J., Monger, T.G. and Parson, W.W. (1975) Biochim. Biophys. Acta. 408, 189-199.
13. Clayton, R.K., and Wang, R.T. (1971) Methods Enzymol. 23, 696-704.
14. Rentzepis, P.M., Topp, M.R., Jones, R.P. and Jortner, J. (1970) Phys. Rev. Lett. 25, 1742.
15. Rentzepis, P.M., Jones, R.P., Jortner, J. (1973) J. Chem. Phys. 59, 766.
16. Dutton, P.L., Petty, K.M., Bonner, H.S. and Morse, S.D. (1975) Biochim. Biophys. Acta. 387, 536-556.
17. Clayton, R.K. and Yamamoto, T., Proceedings of the American Soc. for Photobiol. Meet. at Denver, Feb. 1976, p. 62.
18. Prince, R.C. and Dutton, P.L. (1976) Arch. Biochem. Biophys. 172, 329-334.
19. Petty, K.M. and Dutton, P.L. (1976) Arch. Biochem. Biophys. 172, 335-345.
20. Petty, K.M. and Dutton, P.L. (1976) Arch. Biochem. Biophys. 172, 346-353.
21. Prince, R.C. and Dutton, P.L. (1975) Biochim. Biophys. Acta 387, 609-613.