

THE PRIMARY CHARGE-SEPARATION RATE IN ISOLATED PHOTOSYSTEM II REACTION CENTER COMPLEX

MICHAEL R. WASIELEWSKI, DOUGLAS G. JOHNSON, GOVINDJEE\*, CHRISTOPHER PRESTON\*\* AND MICHAEL SEIBERT\*\*, ARGONNE NATIONAL LABORATORY, ARGONNE, ILLINOIS 60439; \*UNIVERSITY OF ILLINOIS, URBANA, ILLINOIS, 61801, \*\*SOLAR ENERGY RESEARCH INSTITUTE, GOLDEN, COLORADO 80401, U.S.A.

1. INTRODUCTION

The primary charge-separation act in isolated bacterial reaction center (RC) complex occurs in 2.8 ps at room temperature and 0.7-1.2 ps at 10K [see refs. in 1]. Because of similarities between the bacterial and photosystem II (PSII) RC's, it is of considerable interest to obtain analogous charge-separation rates in the higher plant system. Although the original isolation of the PSII RC was an important advance [2], the complex was too unstable [3-5] for femtosecond transient absorption spectroscopy. Recently, we have reported several procedures that stabilize the PSII RC complex in both the dark and the light [3,6]. One of these procedures produces material that is sufficiently stable to survive the extensive signal averaging required to measure the primary charge-separation act [1,7].

2. PROCEDURES

The PSII RC complex was isolated from market spinach by the Nanba Satoh procedure [2]. This material was subsequently stabilized by adding a polyethylene glycol (PEG) precipitation step [3,6] and removing O<sub>2</sub> from the sample material with an enzymatic O<sub>2</sub>-scrubbing system [6]. Femtosecond transient absorption techniques and equipment have been described elsewhere [1,7].

3. RESULTS AND DISCUSSION

Ground state absorption spectra and electron transport rates indicate that the PSII RC material was sufficiently active to attempt fast kinetic studies [1,3,6,7]. Figure 1 shows transient absorption difference spectra of isolated PSII RC complex at both 277 and 15K. Solid curves represent open RC's (Pheo in the oxidized state) and dashed curves represent closed RC's (Pheo pre-reduced with dithionite and light prior to a flash). Note that closing the RC's results in loss of the 500-550 nm and 750-850 nm transient absorption features characteristic of P680<sup>+</sup>-Pheo<sup>-</sup>.

The kinetics at 820 nm (Fig. 2) are representative of the only unambiguous spectral region in which the formation of P680<sup>+</sup>-Pheo<sup>-</sup> is not convoluted with the appearance and decay of the lowest excited singlet state of P680 (<sup>1</sup>\*P680) or other pigments. A monophasic exponential increase is observed with a best fit of  $\tau = 3.0 \pm 0.6$  ps (277K) and  $\tau = 1.4 \pm 0.2$  ps (15K).

M. Baltscheffsky (ed.), *Current Research in Photosynthesis*, Vol. I, 451-454. © 1990 Kluwer Academic Publishers. Printed in the Netherlands.

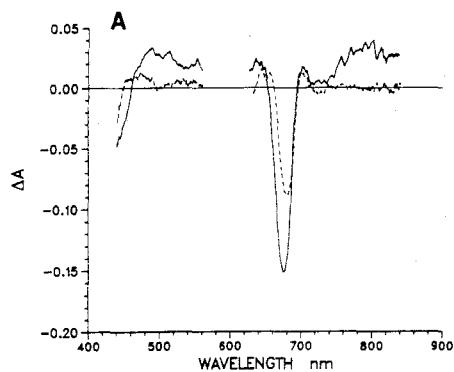
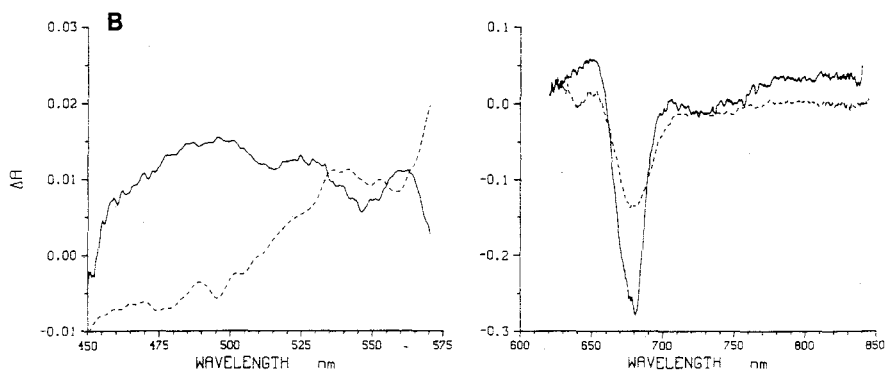


FIGURE 1. Transient absorption spectra of open PSII RC's (solid curve) or of closed PSII RC's pre-treated with 1.5 mM sodium dithionite and 15  $\mu$ M methyl viologen (dashed curve). All traces occur 10 ps following a 100- $\mu$ J, 500-fs laser flash at 610 nm. Filters needed to reject residual 610-nm light following continuum-probe light generation block the 570- to 620-nm wavelength range. A. 277K B. 15K. (After [1] and [7]).



In examining the kinetics of the absorption changes across the transient absorption difference spectra of  $P680^+-Pheo^-$  at 277K, we found that 650 nm is a near isosbestic point for the difference between the absorbances of  $P680^+-Pheo^-$  and  $P680-Pheo$ . At this wavelength (data not shown), the absorption rises sharply within the time resolution of the instrument and decays with a  $\tau = 2.6 \pm 0.6$  ps. We ascribe this absorption increase to  $^1P680$  based on spectral arguments [1,7] and the fact that its decay time agrees well with the rise time of  $P680^+-Pheo^-$  (Fig. 2). The lifetime of  $^1P680$  can also be obtained by measuring the decay of its stimulated emission. At 15K stimulated emission arising from the Q (0,1) vibronic band of  $^1P680$  can be observed at 740 nm (data not shown). The appearance of the bleach is instrument limited followed by a single decay with  $\tau = 1.2 \pm 0.4$  ps. Again the decay of  $^1P680$  (i.e., its stimulated emission) is in good agreement with the appearance of  $P680^+-Pheo^-$  at 15K (Fig. 2B). These data are also in agreement with hole-burning data on PSII RC complexes [8].

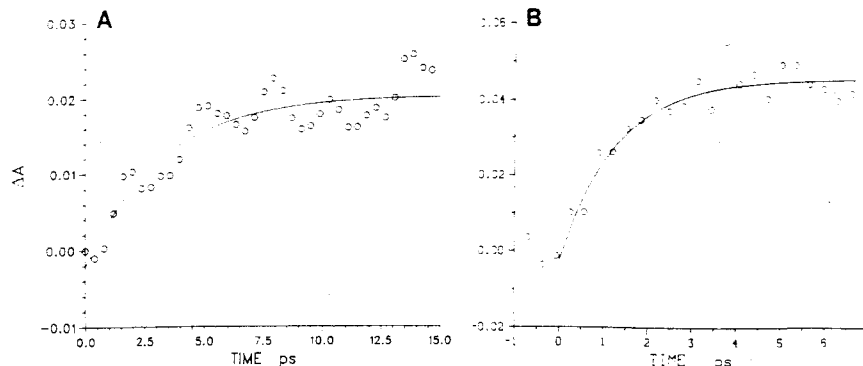
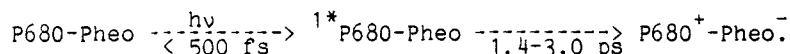


FIGURE 2. Transient absorption changes at 820 nm for PSII RC's after a 100- $\mu$ J, 500-fs laser flash at 610 nm. A. 277K B. 15K. (After [1] and [7]).

Kinetics at other wavelengths (450-770 nm) reflect mixtures of both the above mentioned components. Since at 15K we observe partial resolution of two peaks in the  $Q_y$  bands of the PSII RC pigments, Figs. 3 and 4 focus on the kinetics of the bleach and recovery in this region. Fig. 3 shows the spectral changes that occur immediately after the laser flash. The 672 nm bleach is instrument limited while the 682 nm bleach reflects both  $^1P680$  and  $P680^+-Pheo^-$  formation. Figs. 4A and 4B show the recovery of the bleach in open and closed PSII RC's, respectively. Recovery is wavelength dependent, and, in the case of pre-reduced RC's (Fig. 4B), the long recovery component on this time scale is  $^1P680$ . The fact that the 672 nm bleach recovers quickly regardless of the oxidation state of Pheo suggests that this component is not associated with primary charge separation or recombination processes. Similar wavelength-dependent recovery is not observed at 277K.

In summary the absorption data suggest that both P680 and photo-active Pheo in PSII RC's contribute to absorption near 682 nm and as such P680 is a shallow trap for excitation energy from the other pigments contributing to absorption at 672 nm. Furthermore, primary charge separation in PSII is represented by the following reactions:



No evidence for an intermediate acceptor, such as  $Chl^-$ , was obtained at times  $> 500$  fs. The rate of charge separation increases by a factor of two when the temperature is lowered from 277 to 15K, similar to results observed previously with bacterial RC's. Finally, the apparent rapid recovery of the 672 nm band ( $\tau \sim 25$  ps) suggests the presence of a second photophysical process in PSII RC's at 15K (but not 277K) that is not coupled to primary charge separation.

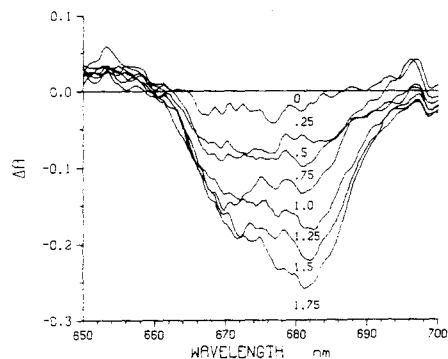
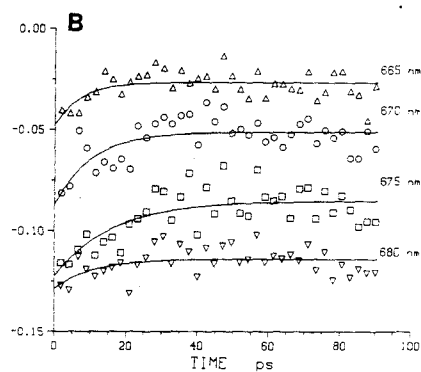
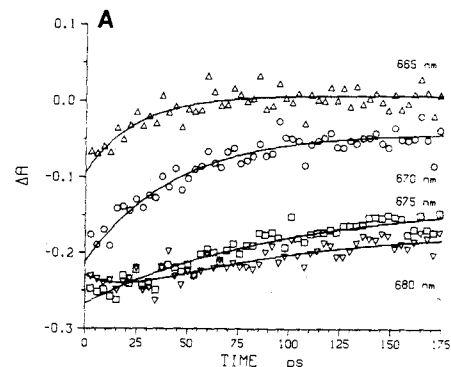


FIGURE 3. Transient absorption spectra for untreated PSII RC's at 0.25 ps intervals at 15K. (After [7]).

FIGURE 4. Transient absorption changes at 665, 670, 675, and 680 nm following a laser flash. A. Open PSII RC's. B. Closed PSII RC's. Temperature, 15K. (After [7]).



#### ACKNOWLEDGEMENTS

This work was supported by the Chemical Sciences (MRW) and Energy Biosciences (M.S.) Divisions of the Office of Basic Energy Sciences, U.S. Department of Energy, and by a McKnight interdisciplinary grant (G).

#### REFERENCES:

1. Wasielewski, M.R., Johnson, D.G., Seibert, M., and Govindjee (1989) PNAS, USA 86, 524-528.
2. Nanba, O., and Satoh Ki. (1987) PNAS, USA 84, 109-112.
3. Seibert, M., Picorel, R., Rubin, A.B., and Connolly, J.S. (1988) Plant Physiol. 87, 303-306.
4. Akabori, K., Tsukamoto, H., Tsukihari, J., Nagatsuka, T., Motokawa, O., and Toyoshima, Y. (1988) Biochim. Biophys. Acta 932, 345-357.
5. Chapman, D.J., Gounaris, K., and Barber, J. (1988) Biochim. Biophys. Acta 933, 423-431.
6. McTavish, H., Picorel, R., and Seibert, M. (1989) Plant Physiol. 89, 453-456.
7. Wasielewski, M.R., Johnson, D.G., Govindjee, Preston, C., and Seibert, M. (1989), Photosynthesis Res., in press.
8. Jankowiak, R., Tang, D., Small, G.J., and Seibert, M. (1989), J. Phys. Chem. 93, 1649-1654.