PRIMARY CHARGE SEPARATION IN ISOLATED PHOTOSYSTEM II REACTION CENTERS

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1. INTRODUCTION

Primary charge-separation in isolated bacterial reaction center (RC) complex occurs in 2.8 ps at room temperature and 0.7-1.2 ps at 10 K. Because of similarities between the bacterial and photosystem II (PSII) RCs [1], it has been of considerable interest to obtain analogous charge-separation rates in the higher plant system. Our previous femtosecond transient absorption studies used PSII RC material stabilized with PEG (suspended in 0.04% Triton X-100) or by exchanging dodecyl maltoside (DM) for Triton in the isolation procedure. These materials gave charge-separation 1/e times of 3.0 ± 0.6 ps at 4°C and 1.4 ± 0.2 ps at 15 K [2-4] based on the risetime of transient absorption kinetics at 820 nm. These values were thought to represent the time required for formation of the $P680^+$-$Pheo^-$ state. Recent results of Hastings et al.[5], obtained at high data acquisition rates and low flash intensities, suggest that the $Pheo^-$ state may form more slowly. In light of this work, we have carried out additional time domain studies of both electron transport and energy transfer phenomena in stabilized DM PSII RCs at room temperature. We used a 1-kHz repetition rate femtosecond transient absorption spectrometer with a 200 fs instrumental time resolution and compared the results with those obtained by others using frequency domain hole-burning techniques [6,7].

2. PROCEDURES

The PSII RC complex was isolated from market spinach by a modification of the Nanba-Satoh procedure [1] in which the material was removed from the column in the presence of 0.03% DM instead of Triton. The DM RCs contained a 6:2:1:2 ratio of Chl:Pheo:Cyt b-559:ß-carotene (confirmed by spectroscopic and HPLC techniques in the laboratory of Dr. R. Picorel). We have shown previously that DM stabilizes isolated PSII RC [8,9] and does not affect the spectral properties of the complex as is the case with Triton [6,7,10]. Further stabilization of the complex for spectroscopic studies was accomplished by removing O_2 from the sample material with an enzymatic O_2-scrubbing system [9]. Femtosecond transient absorption equipment has been described elsewhere [2,3]. The laser system with a 10 Hz flash repetition rate uses 500-fs, 610 nm excitation pulses with a 500-fs instrument response time, while the 1-kHz flash repetition rate laser system uses 160-fs, 585 nm excitation pulses with a 200-fs instrument response time. Control experiments using this apparatus to excite Chl in pyridine did not elicit multiphoton events.

3. RESULTS AND DISCUSSION

Figure 1 shows transient absorption difference spectra of isolated DM PSII RC complex at different temperatures and data acquisition rates. The 7 K spectrum (10 Hz) has better resolved troughs at 672 and 683 nm than previously reported [3], because DM was exchanged for Triton in the sample. The 297 K spectrum of open RCs (1-kHz; solid curve) is shifted to 680 nm from 674 reported previously [2], again due to the elimination of Triton from the sample. The 297 K spectrum of closed RCs (1-kHz; dashed curve; Pheo was pre-reduced with dithionite and light prior to exposure to laser flashes, thus preventing primary charge separation) is quite similar to our previous results with Triton-solubilized material [2]. The 680 nm bleach is due to the lowest

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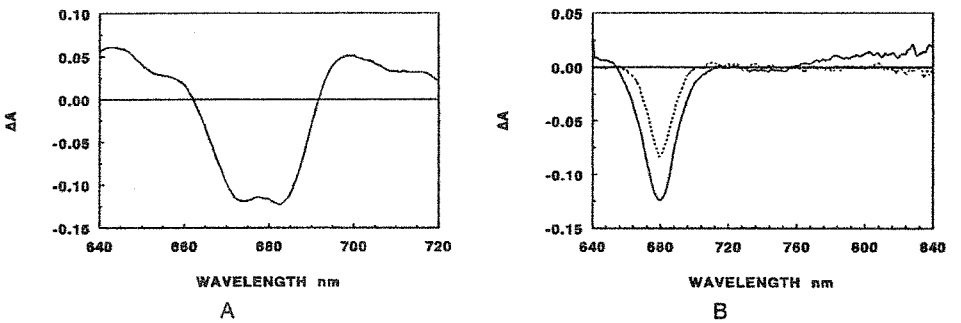

Figure 1 - Transient absorption spectra at 10 ps of open (—) DM PSII RCs. A. 100-μj, 1 ps, 10-Hz flashes at 610 nm; 7 K; minima, 672 and 683 nm. B. 8-μj, 160-fs 1-kHz flashes at 585 nm; 297 K; minimum, 680 nm, closed (---) RCs.

excited singlet state of P680 (^{1}*P680) and occurs in < 200 fs after a flash. Note that closing the RCs results in the loss of the 750-850 nm transient absorption features attributed to charge separation. Similar results have been obtained at data acquisition rates of 10 Hz at 277 K.

The kinetics at 820 nm in open RCs (Fig. 2A) is representative of the only unambiguous spectral region in which the formation of both $P680^+$ and $Pheo^-$ is not convoluted with the appearance and decay of ^{1}*P680 (Fig. 1B) or other pigment excited states. A monophasic exponential increase is observed with a best fit of τ = 3.3 ± 0.5 ps (297 K). The risetime for DM PSII RC at 7 K is 1.4 ± 0.2 ps (not shown), also reported previously [3]. The value at low temperature is consistent with that obtained from transient hole burning studies of P680 [7]. In this work, the width of the zero phonon hole (ZPH) was related to the lifetime of ^{1}*P680 by the uncertainty principle and corresponds to a value of 1.9 ps. The presence of detergent does not affect the width of ZPH [7] and hence the lifetime. Thus there is a clear correlation between the τ of ^{1}*P680 determined by the frequency domain technique and the transient absorption risetime at 820 nm attributed to charge separation. At a minimum then, the disappearance of ^{1}*P680 correlates with the appearance of $P680^+$.

In Figure 2B, we demonstrate that part of the immediate transient absorption increase at 545 nm also recovers with a τ = 3.5 ± 0.5 ps (297 K). The absorption decrease is interpreted as Pheo reduction. Thus our hole burning and transient absorption studies are consistent with the formation of the $P680^+$-$Pheo^-$ state from ^{1}*P680 in about 3 ps at 297 K and in 1.4-1.9 ps at 4-7 K. *The appearance kinetics of both ions in the charge separation reaction are the same. This equivalence is required for the formation of $P680^+$-$Pheo^-$ from a common precursor state.* In this case the precursor state is ^{1}*P680. At present our data provides no evidence for the formation of Chl^- as an intermediate preceding Pheo reduction.

Figure 3 shows recovery kinetics of the rapid transient absorption decreases at 665 nm and 685 nm. The recovery of the bleach at 665 nm can be fit either with a 24 ± 2 ps one component fit or a 12 ps and 50 ps two-component fit (not shown), while no slow recovery (on the 10-50 ps timescale) is seen at 685 nm. The 24 ps component that we observe can be compared to the 21-ps kinetic component observed by Hasting's et al.[5]. We have observed this phenomenon before at helium temperature [3], and have concluded that it is the result of energy transfer from the accessory pigments of the PSII RC unconnected from direct primary charge

separation. Persistent hole burning studies of PSII RCs [6,7], also at helium temperature, show that light absorbed by the accessory pigment band (probably monomeric Chl at about 670 nm) results in the formation of a broad satellite band centering at 681.6 nm (active Pheo). The satellite

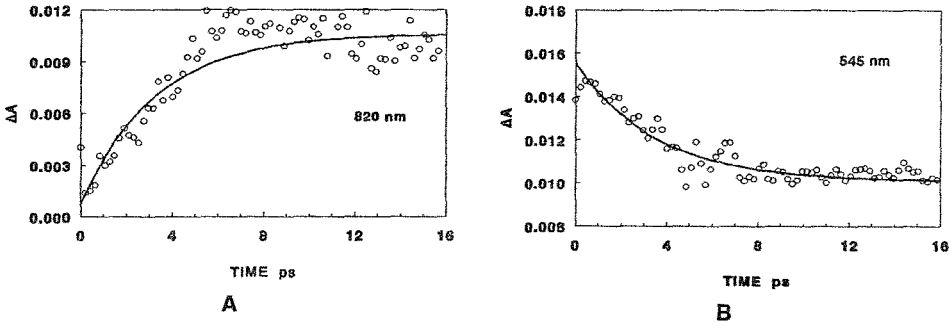

Figure 2. - Transient absorption changes for open PSII RCs at 297 K after 200-fs laser flashes at 1-kHz. A. Kinetics at 820 nm is fit to a τ = 3.3 ± 0.5 ps. B. Kinetics at 545 nm shows a rapid increase due to excited state formation and a slower bleach due to pheo reduction. The latter can be fit with a τ = 3.5 ± 0.5 ps.

band must be formed as a result of energy transfer and measurements of the width of the ZPH indicate that the transfer time is about 12 ps. When persistent holes are burned directly into the 680 nm region, the satellite peak disappears and the ZPH hole indicates an excited state lifetime of around 50 ps. This latter phenomenon has been interpreted as energy transfer from Pheo to P680* [6,7].

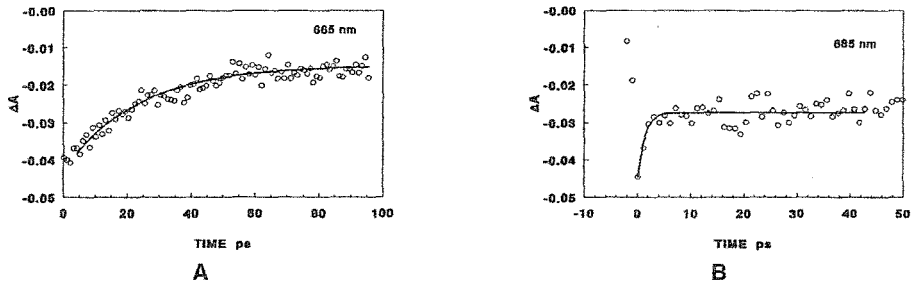

Figure 3. - Transient absorption changes at A. 665 nm and B. 685 nm for open DM PSII RCs at 297 K after 160-fs laser flashes at 585 nm (1-kHz).

4. CONCLUSIONS

The reaction ^{1*}P680-Pheo -> P680^{+}-Pheo^{-} within PSII RCs occurs with ca. a 3 ps time constant as determined by us previously[2] and confirmed in this work with stabilized DM PS II RCs. This time constant has been confirmed by observing the formation of *both* P680^{+} and Pheo^{-}. In addition, we have observed a kinetic component of about 20 ps in the transient absorption recovery data that we interpret as due to energy transfer within the PSII RC. This energy transfer

process is not directly connected to the primary charge separation reaction. This interpretation is strongly supported by photochemical hole-burning data. Perhaps the ca. 20 ps component in the kinetics of Hastings et al.[5] should be reexamined in light of these considerations.

5. ACKNOWLEDGEMENT

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