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Rates of Primary Electron Transfer Reactions in the Photosystem I Reaction Center Reconstituted with Different Quinones as the Secondary Acceptor

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Rates of sequential electron transfer reactions from the primary electron donor chlorophyll dimer (P700) to the electron acceptor chlorophyll *a*-686 (A_0) and to the secondary acceptor quinone (Q_ϕ) are measured by picosecond absorption spectroscopy in spinach photosystem I (PS I) particles. In the particles, 85−90% of antenna chlorophylls are extracted and the intrinsic phylloquinone (Q_ϕ) is removed and replaced by different quinones. (1) The excited single state of P700, monitored by the absorbance change at 695 nm, appeared with a time constant shorter than 1 ps after excitation with a 605 nm, 1 ps pulse at an intensity of 0.8 or 1.5 photons/reaction center. (2) The formation of a $P700^+A_0^-$ state, which was monitored by the absorbance change of A_0 at 685 nm, occurred with a time constant (τ_1) of 8 ps. The $P700^+A_0^-$ state persisted more than 800 ps in the phylloquinone-depleted particles. (3) After the reconstitution of menaquinone-4, phylloquinone, or 2-methyl-1,4-naphthoquinone as Q_ϕ, the transition from the $P700^+A_0^-Q_\phi$ to $P700^+A_0Q_\phi^-$ state (monitored by the recovery of the A_0 absorption band) occurred with time constants (τ_2) of 23, 23, or 34 ps in the particles with an antenna size of 30 chlorophylls/P700. (4) In the PS I particles with an antenna size of 16 chlorophylls/P700, a similar τ_1 of 11 ps and a τ_2 of 31 ps were observed when menaquinone was reconstituted as Q_ϕ. (5) Indication for the absorbance change of the chlorophylls, which may function as the intermediate electron acceptor between P700 and A_0 or between A_0 and Q_ϕ, was not obtained.

1. Introduction

The reaction center (RC) pigment−protein complexes of photosynthetic organisms undergo light-induced charge separation and convert light energy to the electrochemical potential. In the isolated RC complex of a purple photosynthetic bacterium, *Rhodobacter sphaeroides*, the electron transfer reactions from the donor bacteriochlorophyll *a* dimer (P860) to a bacteriopheophytin *a* (BH) and from BH^- to a ubiquinone (Q_A) have been shown to proceed with time constants of 3.5 and 220 ps, respectively, at room temperature.[1] The photosystem II (PS II) RC complex of plant chloroplasts or cyanobacteria has a structure essentially homologous to that of purple bacterial RC complexes.[2,3] The electron transfer reaction from the donor chlorophyll *a* (P680) to a pheophytin *a* (H) is reported to proceed with a time constant of 3[4] or 21[5] ps, and that from H^- to a plastoquinone (Q_A) is reported to proceed with a time constant of 350 ps.[6]

The polypeptides and electron transfer components of photosystem I (PS I) RC complexes of plant and cyanobacteria are different from those of purple bacteria/PS II RCs.[7−9] The PS I RC contains about 100 molecules of antenna chlorophylls and four electron carriers: the primary electron donor chlorophyll *a* dimer (P700), the primary acceptor chlorophyll *a* (A_0), the secondary acceptor quinone (initially designated A_1[8] and designated Q_ϕ here), and the tertiary acceptor iron sulfur cluster (F_X).[8] F_X reduces the other iron sulfur clusters, F_A and F_B, that are contained on the polypeptide bound to the RC surface.[10] The Q_ϕ in the native plant PS I RC is one of the two phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone, PhyQ) molecules contained in the RC complex.[8,11] A recent X-ray crystallographic study with a 6 Å resolution in PS I RC indicated the locations of P700, other chlorophylls, and the iron sulfur clusters.[12] However, the locations of A_0 and Q_ϕ are still tenative.

Previously, an apparent time constant of the formation of $P700^+A_0^-$ (τ_1 in Figure 1) was measured to be 13.7 ps,[13] and the time constant of the electron transfer from A_0^- to Q_ϕ (τ_2 in Figure 1) was estimated to be 32 ps[14] by the transient absorption measurements in the isolated PS I particles. The intrinsic time constant of the electron transfer from the excited singlet state of P700 (P700*) to A_0 is estimated to be equal to or smaller than τ_1 if we consider the effect of energy transfer between P700 and other chlorophylls. Very recent studies on the thylakoid membranes of a cyanobacterial mutant indicated τ_2 to be 21 ps by measurements with very strong excitation (at 4−8 photons/P700) conditions.[15] These τ_1 and τ_2 values, however, were obtained with different types of PS I preparations or under different experimental conditions and have never been measured sequentially (see also Table 1). A few recent works of picosecond spectroscopy, on the other hand, failed to detect A_0^-.[16,17]

The extraction of PhyQ was shown to suppress the normal electron transfer from A_0^- to iron sulfur clusters[9,11,18−23] and to enhance the charge recombination between A_0^- and $P700^+$ with a time constant of 47−53 ns.[20−23] Reconstitution of PhyQ or other quinones recovered the electron transfer from A_0^- to F_X.[9,11,24,25] However, these studies did not measure τ_2 directly with sufficient time resolution.

In the present study, the τ_1 and τ_2 values were determined in the PS I particles in which PhyQ was extracted by diethyl ether and PhyQ, menaquinone-4 (MK, 2-methyl-3-prenyl-1,4-naph-

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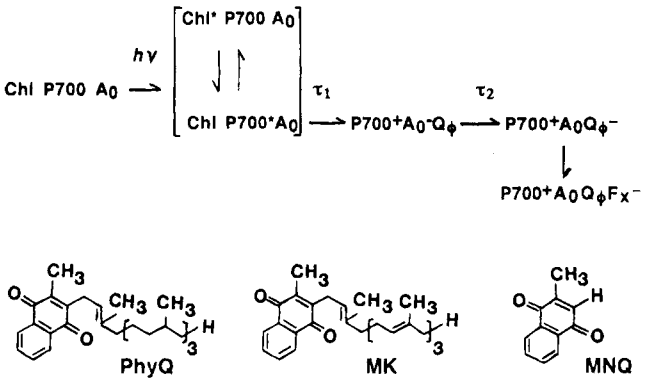

Figure 1. Reaction scheme in PS I reaction center. τ_1 and τ_2 represent the apparent time constants for the primary charge separation and the secondary electron transfer, respectively. Molecular structures of phylloquinone (PhyQ), menaquinone-4 (MK), and 2-methyl-1,4-naphthoquinone (MNQ) that were used to reconstitute Q_ϕ are also shown.

thoquinone), or 2-methyl-1,4-naphthoquinone (MNQ) was reconstituted as Q_ϕ (Figure 1). The extraction procedure also reduced the number of antenna chlorophylls.

2. Experimental Section

Lyophilized PS I particles, which were obtained by treating spinach chloroplasts with digitonin, were twice extracted with a 4:6 or 2:8 mixture of dried and water-saturated diethyl ether to obtain PS I particles with a total chlorophyll/P700 mole ratio (Chl/P700) of 30 or 16, respectively. This procedure removed phylloquinones, carotenoids, and about 85−90% of the antenna chlorophylls.[18,19] P700, A_0, F_X, F_B, and F_A were not destroyed by the extraction.[11,19] The extracted particles were dispersed in 50 mM tris(hydroxymethyl)aminomethane-Cl buffer (pH 7.5) containing 0.05% (v/v) Triton X-100 to give a final P700 concentration of 0.5 μM. All these extraction procedures were done at 0−4 °C. To reconstitute PhyQ, MK, and MNQ, as Q_ϕ into the particles, each quinone dissolved in dimethyl sulfoxide was added to the reaction mixture to give a final concentration of 5, 5, or 20 μM and incubated for a day at 0 °C as reported.[18]

Pump−probe spectra were measured using a double-beam spectrometer and an amplified picosecond dye laser as described previously.[26] A pulse at 605 nm with a full width at half-maximum of 1 ps was used at a repetition rate of 10 Hz. A small fraction of the pulse was used to excite the sample, the remainder to generate a white light continuum in a 2:1 H_2O/D_2O mixture for probing. Polarization of the beams was at parallel. The difference in arrival times (400−500 fs) of the probe beam to the sample in the monitored spectral region (660−720 nm) was not corrected. Pump and probe beams were overlapped at an angle of 5° with beam diameters of about 2 and 0.5 mm, respectively, in a flow cell of 10 mm light path. The beams were imaged onto the slits of two identical 25 cm monochromators and detected by photodiode arrays (Hamamatsu Photonics C2326-22A) with a wavelength resolution of 1.5 nm. The absorbance at the 676 nm peak of the sample was adjusted to be 1.2. Signals from 300 laser shots were accumulated to obtain one transient spectrum. The instrument response time, which was measured by the 10−90% bleaching time of Oxazine 750 in 1-chloronaphthalene in the same cell, was 2 ps.

The sample was placed in a flow cell connected to a reservoir in ice and circulated by a peristatic pump to avoid denaturation. Transient spectra of the sample in the presence of 0.2 mM ferricyanide (P700-preoxidized conditions) were measured first. Then, ascorbate and phenazine ethosulfate were added to give final concentrations of 10 mM and 2 μM, respectively, to reduce $P700^+$, and the measurements were repeated (P700-neutral conditions).

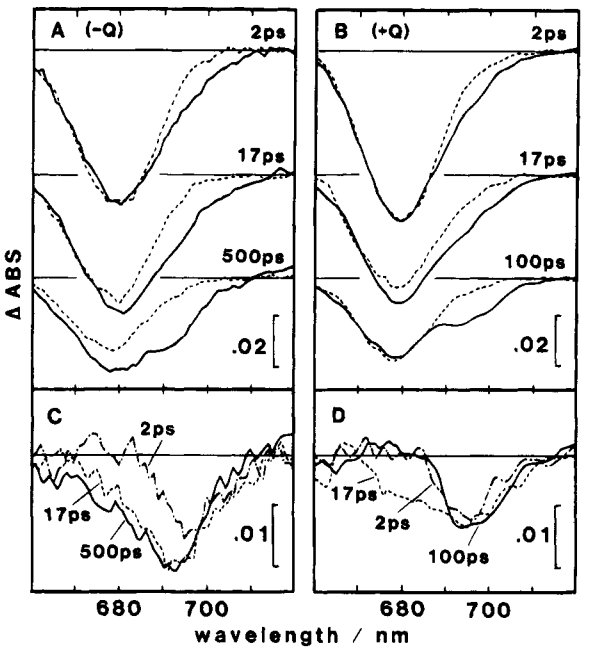

Figure 2. Time-resolved difference absorption spectra induced by the 1 ps flash in the 30 Chl/P700 particles. A and C, measurements in the quinone-depleted particles. B and D, measurements in the menaquinone-reconstituted particles. The transient spectra measured under the P700-neutral (—) and -preoxidized (- - -) conditions are shown in A and B. The delay times are shown in the figures. C and D, neutral-minus-preoxidized difference spectra at the same delay time. Thin horizontal lines indicate the zero levels. Scales for absorbance change are shown in the figures. Excitation intensity was set at 1.5 photons/RC.

3. Results

3.1. Transient Absorption Spectra of the 30 Chl/P700 Particles in the Absence of Phylloquinone and the Effect of Quinone Reconstitution. A series of transient absorption spectra was measured in the 30 Chl/P700 PS I particles in which the intrinsic PhyQ (Q_ϕ) was extracted. In this preparation, the absorbance changes of P700 and A_0 can be typically monitored at 695 and 686 nm, respectively.[15,23,27−30] Under the P700-preoxidized conditions (broken lines in Figure 2A), excitation induced a negative absorbance change. The absorbance change showed a peak at 678−679 nm with a full bandwidth at e^{-1} of the maximum (FW e^{-1} M) of 560 (±30) cm^{-1} (Figure 2A). The wide bandwidth indicates nonspecific excitations of different chlorophyll forms. After the full development of the signal within 1 ps, about 50% of the signal decayed with a time constant ($t_{1/e}$) of 15 ps, and the remaining fraction showed almost no decay up to the available maximum delay time of 800 ps.

Transient absorption spectra were also measured under the P700-neutral conditions (solid lines in Figure 2A). These spectra resemble those measured under the P700-preoxidized conditions except the extra bleach between 670 and 710 nm. The difference between the spectra measured under the P700-neutral and -preoxidized conditions at the same delay time (the difference between the solid and dashed lines in Figure 2A) is shown in Figure 2C. The neutral-minus-preoxidized difference spectrum at 2 ps shows a peak at 695 nm with a bandwidth of 300 ± 30 cm^{-1} (FW e^{-1} M). The spectrum can be ascribed to the depletion of the ground state of P700, judging from its peak wavelength and bandwidth. This seems to represent the formation of P700*. No concomitant absorbance change of the

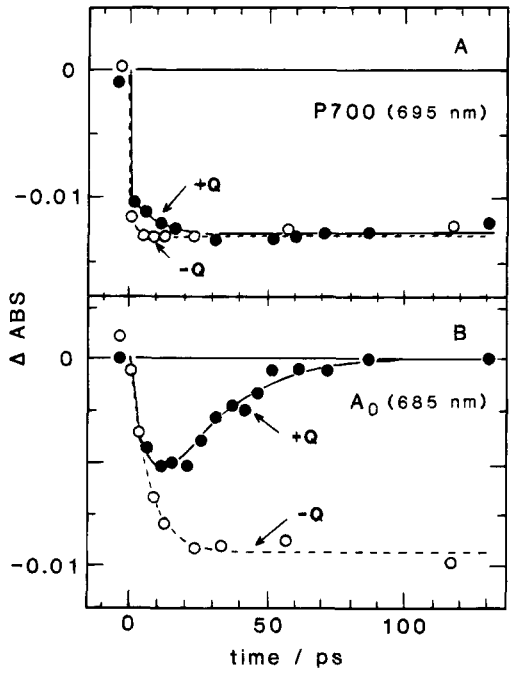

Figure 3. Reaction kinetics of P700 (A) and A_0 (B) in the quinone-depleted and menaquinone-reconstituted PS I particles. Absorbance changes P700 and A_0 were measured at 695 and 685 nm, respectively, in the 30 Chl/P700 particles in which PhyQ was extracted (○) or menaquinone was reconstituted (●). The averages for the data points between 695 ± 2 and 685 ± 2 nm in Figure 2C,D at each delay time were plotted. In A, the magnitude of P700 in the MK-reconstituted particles is normalized to give the same final extent with that in the quinone-depleted particles. The curves are arbitrarily drawn. In B, the same normalization factor was used to adjust the kinetics in the MK-reconstituted particles. The fitting curves are the convolutions of the instrument response and the assumed reaction kinetics (see text).

other chlorophylls, which can be ascribed to the electron acceptor, was detected at 2 ps. From 2 to 17 ps, a bleach at around 685 nm developed in addition to that at 695 nm. The bleach can be ascribed to the reduction of the primary electron acceptor A_0, judging from its peak wavelength[15,23,27-30] (see Figure 4C for the difference spectrum of A_0^- minus A_0, which is hereafter denoted A_0^-/A_0). The simultaneous bleaches of P700 and A_0 at 17 ps suggest the formation of the $P700^+A_0^-$ biradical state. No indication for the electron acceptor other than A_0 was detected. The neutral-minus-preoxidized spectra at times between 2 and 17 ps (not shown) were explained as the sum of $P700^+/P700$ and A_0^-/A_0 difference spectra.

Transient absorption spectra were also measured in the particles reconstituted with MK as Q_ϕ (Figure 2B). The spectral shapes measured under the P700-preoxidized conditions (dashed lines) are similar to those measured in the quinone-depleted particles. Under P700-neutral conditions, extra bleaches were observed at 670−710 nm (solid lines in Figure 2B). In the neutral-minus-preoxidized spectra (Figure 2D), the bleach at 695 nm (P700*) almost fully developed at 2 ps. The bleach at 685 nm due to the formation of A_0^- was seen at 17 ps, as seen in the PhyQ-depleted particles, and then disappeared at 100 ps. Only the bleach around 695 nm remained at 100 ps. The spectrum at 100 ps resembles the difference spectrum of $P700^+/P700$. This suggests that the transition from the $P700^+A_0^-MK$ to $P700^+A_0MK^-$ state takes place from 17 to 100 ps.

The extents of absorbance changes at 685 and 695 nm in the neutral-minus-preoxidized spectra in Figure 2C,D are plotted against time (Figure 3) to monitor the absorbance changes of P700 and A_0, respectively. The bleach of P700 developed to 80% of its final extent within 1 ps (indistinguishable from the instrument response), both in the presence and absence of MK (Figure 3A). This kinetics is explained by the rapid formation of P700* and the subsequent formation of $P700^+$ (P700* and $P700^+$ cannot be distinguished at 695 nm). The kinetics at 685 nm in the PhyQ-extracted particles was fitted to the convolution of the instrument response function and the single-exponential rise with a time constant of 8 ± 2 ps (Figure 3B). The kinetics at 685 nm in the MK-reconstituted particles, on the other hand, was fitted by the exponential rise and decay with time constants of 8 ± 2 and 23 ± 5 ps, respectively (Figure 3B). These results suggest a 8 ps time constant (τ_1) for the transition from $P700^*A_0$ to $P700^+A_0^-$ and a 23 ps time constant (τ_2) from $P700^+A_0^-MK$ to $P700^+A_0MK^-$. The τ_2 value was about 3 times larger than the τ_1 value so that only about 60% of the full extent of A_0^- was accumulated at the peak amplitude in the MK-reconstituted particles. The kinetics also indicates that MK functions as Q_ϕ in 95−100% of PS I reaction centers.

PhyQ and MNQ were reconstituted as Q_ϕ, and the depletion and recovery of absorption bands of P700 and A_0 were also measured (Table 1). The τ_1 value was unaffected by the quinones. In the particles reconstituted with PhyQ, about 65% of the total $P700^+A_0^-$ state that was formed by the excitation pulse turned into the $P700^+A_0PhyQ^-$ state with a τ_2 value of 23 ± 5 ps. The rest of the reaction centers did not show decay of A_0^-. In the MNQ-reconstituted particles, about 55% of $P700^+A_0^-$ decayed with a τ_2 of 34 ± 7 ps. Some portions of reaction centers were not reconstituted with these quinones under the present experimental conditions.

These results are consistent with previous measurements in which the reconstituted quinones restored the electron transfer from A_0^- to F_X.[9,11,21,24] The fast τ_2 values confirm that these quinones function as the electron acceptor at the Q_ϕ site and not as the exogenous acceptors.

3.2. Reaction of A_0 in the 16 Chl/P700 Particles Reconstituted with MK. Effects of reduction of antenna chlorophyll size on the τ_1 and τ_2 values were studied in the 16 Chl/P700 particles that also lacked PhyQ and retained all the other electron carriers. Transient spectra measured in the MK-reconstituted particles are shown in Figure 4A. The spectra under the P700-neutral conditions show large extra bleaches compared to those measured under the P700-preoxidized conditions (Figure 4A). Relative extents of the extra bleaches were larger than those seen in the 30 Chl/P700 particles in Figure 2. This seems to reflect a lower probability of the excitation of antenna chlorophylls in this preparation. In the bottom panel of Figure 4A, a transient spectrum measured at 400 ps under the P700-neutral condition in the quinone-depleted particles is also shown (dashed and dotted line). The neutral-minus-preoxidized difference spectra at 1, 17, and 400 ps in Figure 4B suggest the formation of P700*, $P700^+A_0^-MK$, and $P700^+A_0MK^-$ states at these delay times in more than 90% of PS I particles.

The τ_1 for the bleach of A_0 was determined to be 11 ± 3 ps in the absence and presence of MK (Table 1). The τ_1 value is similar to the 8 ± 2 ps τ_1 observed in the 30 Chl/P700 particles within the error limit. The τ_2 value was estimated to be 31 ± 6 ps and is equal to or a little longer than that determined in the 30 Chl/P700 particles.

3.3. Differences Spectrum of A_0^-/A_0. The difference spectrum of A_0^-/A_0 was calculated in two different ways from the transient spectra measured in the 16 Chl/P700 particles. First, the neutral-minus-preoxidized difference spectrum in Figure 4B of the $P700^+A_0MK^-$ state at 400 ps was subtracted from that of the $P700^+A_0^-MK$ state at 17 ps in the MK-reconstituted particles (the dashed line in Figure 4C). Second, the difference was calculated between the transient spectra in the presence and

TABLE 1: Apparent Time Constants for the Formation of $P700^+A_0^-$ and the Transition from the $P700^+A_0^-$ to $P700^+A_0Q_\phi^-$ State (τ_2) Obtained by the Picosecond Difference Absorption Spectroscopy in Various PS I Preparations

preparations (Chl/P700 ratio)	Q_ϕ	τ_1 (ps)	τ_2 (ps)	references
PS I particles				
ether extraction				
(30)	no added quinone	8 ± 2	nd*	this work[a]
	MK	8 ± 2	23 ± 5	
	PhyQ	8 ± 2	23 ± 5	
	MNQ	8 ± 2	34 ± 7	
ether extraction				
(16)	no added quinone	11 ± 3	nd*	this work[b]
	MK	11 ± 3	31 ± 6	
ether extraction				
(12)	no added quinone	6.5	nd*	Kumazaki *et al.*[31,c]
(9−12)	MNQ	nd	150	Kim *et al.*[26]
Triton treatment				
(28)	native PhyQ	<40	200	Shuvalov *et al.*[29]
(70−100)	native PhyQ	nd	32	Shuvalov *et al.*[14]
Triton−LDAS−SDS treatment				
(30−40)		13.7	nd	Wasielewski *et al.*[13]
(30−40)	native PhyQ	10	40	Fenton *et al.*[45]
thylakoid membranes from PS II-deficient cyanobacterial mutan				
(100)	native PhyQ	nd	21	Hastings *et al.*[15,d]
	native PhyQ	24−28	nd	Hastings *et al.*[35,e]

[a−e] The excitation intensities of these experiments are 1.5, 0.8, 0.4−0.8, 4−8, and 0.25 photons absorbed/RC, respectively. nd, not determined. (*) No substantial decay was observed until 800 ps.

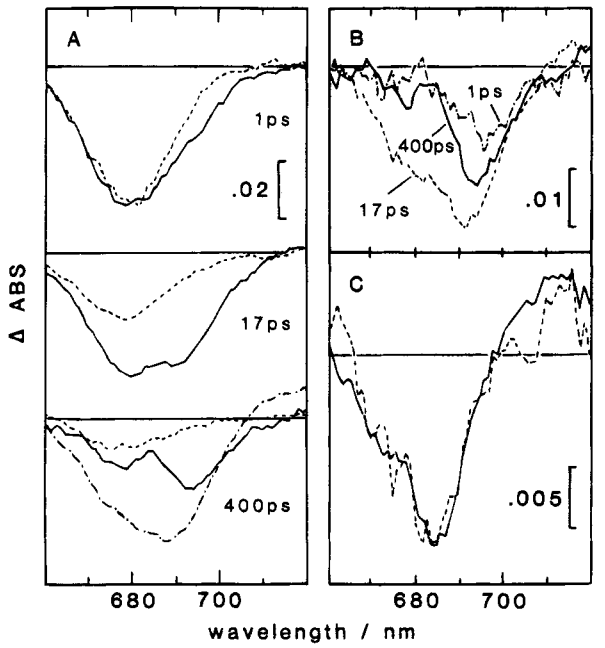

Figure 4. (A) Time-resolved difference absorption spectra in the MK-reconstituted 16 Chl/P700 particles. The spectra measured in the particles under the P700-neutral (—) and -preoxidized (- - -) conditions at the indicated delay times are shown. The spectrum measured in the quinone-depleted particles under the P700-neutral conditions at 400 ps is also added (-·-). (B) P700-neutral and -preoxidized difference spectra. (C) Difference spectrum of A_0^-/A_0 estimated by two methods (see text). The difference between the neutral-minus-preoxidized difference spectra at 17 and 400 ps in B (- - -) is shown. The difference between the transient spectra measured at 400 ps in the MK-reconstituted and quinone-depleted particles under the P700-neutral conditions in A (—) is also added. The magnitude of the latter spectrum is reduced to 60%. The zero levels and the scales of vertical axis are indicated as in Figure 2. Excitation intensity was set at 0.8 photons/RC.

absence of MK at 400 ps under the P700-neutral conditions that are shown in Figure 4A. In the second method, the difference between the Chl*$P700^+A_0^-$MK and Chl*$P700^+A_0$MK$^-$ states is calculated, where Chl* represents the excited state of antenna chlorophylls at 400 ps. The amplitude of the spectrum is reduced to about 60% (the solid line in Figure 4C). Both methods gave almost identical spectra for A_0^-/A_0 with a negative peak at 686 nm, a shoulder at 675 nm, and positive absorbance changes at wavelengths above 700 nm. The first method gives an A_0^-/A_0 difference spectrum at the peak amplitude in the presence of MK. The smaller A_0^-/A_0 difference spectrum obtained by the first method indicates that only 60% of the full extent of A_0^- was accumulated at 17 ps. This agrees with the kinetics with τ_1 and τ_2 obtained above. These A_0^-/A_0 difference spectra are similar to those previously reported.[14,15,23,27−30] The spectrum indicates that P700 and A_0 can be selectively monitored at 695 and 685 nm, respectively, as done in Figure 3. The same procedure in the 30 Chl/P700 particles also gave a similar, but a little more noisy, difference spectrum of A_0^-/A_0.

4. Discussion

We determined the time constants for the bleach (τ_1) and recovery (τ_2) of A_0 (Chl *a*-686) to be 8 and 23 ps, respectively, in the 30 MK-reconstituted Chl/P700 particles. No decay of A_0^- was detected within 800 ps in the PhyQ-depleted particles. The τ_2 value varied depending on the quinones reconstituted as Q_ϕ. These results strongly suggest that A_0^- directly reacts with Q_ϕ and confirm that the $P700^+A_0^-$ state appears with τ_1 and changes into the $P700^+A_0Q_\phi^-$ state with τ_2 as shown in Figures 1 and 5. The features of the primary electron transfer reactions in PS I RC are discussed below on the basis of these time constants.

4.1. Electron Transfer from P700 to A_0. The bleaching of P700 occurred with a time constant shorter than 1 ps after excitation with the 1 ps excitation pulse at intensities of 0.8 and 1.5 photons/RC at 605 nm. The rate of P700 excitation observed is a sum of direct and indirect excitation rates, since the 605 nm pulse excites both P700 and the other chlorophylls (see Figure 1).

Wasielewski *et al.*[13] reported the τ_1 value of 13.7 ps in the analysis of P700 neutral-minus-preoxidized difference spectra (Table 1). The τ_1 values of 8 ps obtained in this study and 6.5 ps obtained recently[31] are about 2 times shorter than their result. The discrepancy may come from the difference in the prepara-

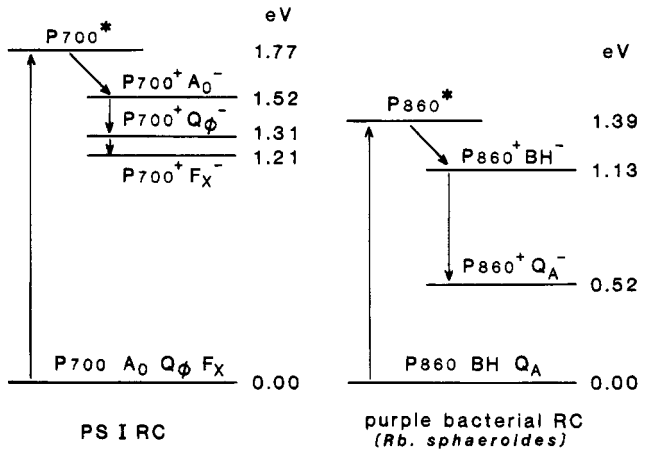

Figure 5. Diagrams of the energy levels and electron transfer steps in the RCs of PS I and purple bacteria (*Rb. sphaeroides*). Horizontal bars indicate the energy levels with respect to the ground state expressed in eV. The energy levels in PS I RC are calculated from the reported redox potentials[8] of P700 (480 mV[42]), A_0 (around −1040 mV[43]), Q_ϕ (around −830 mV[9]), and F_X (−730 mV[44]). The levels in purple bacterial RC are according to ref 39. Major electron transfer pathways are shown by downward arrows.

tions used or the other experimental conditions. The equilibration of excitation energy between P700 and other chlorophylls is expected to affect τ_1 as described by a trap-limited primary charge separation model.[31−34] Thus, the intrinsic time constant for the electron transfer from P700* to A_0 has been estimated to be 3.0 ps previously[31] or 0.43−4 ps.[33−35] Change of antenna size is expected to affect the distribution of excitation energy. The decrease in the antenna size from 30 to 16 Chl/P700, however, only slightly affected the τ_1 in the presented study. Intensity of excitation pulse (Table 1) may also affect τ_1.[31,33−35]

4.2. Electron Transfer from A_0^- to the Reconstituted Quinone. The τ_1 and τ_2 values estimated in the present and previous studies are summarized in Table 1. Similar τ_2 values of 23 ps were estimated for the cases with the reconstituted MK and PhyQ in the 30 Chl/P700 particles. These values are comparable to the τ_2 values of 21 ps estimated in the thylakoid membranes of a cyanobacterial mutant that lacks the PS II reaction center[15] or of 32 ps estimated in a relatively intact PS I preparation containing intrinsic PhyQ.[14] The short τ_2 with MK seems to suggest that its molecular structure and redox potential are similar to those of PhyQ. Kim *et al.* reported a τ_2 of 150 ps with reconstituted MNQ in 9−12 Chl/P700 particles.[27] MNQ gave a shorter τ_2 of 34 ps in this work. The τ_2 was not very much different from that observed with MK or PhyQ. The energy gap between the A_0^-MNQ and A_0MNQ$^-$ states is larger than that between the A_0^-PhyQ and A_0PhyQ$^-$ states by about 50 mV.[9] The small shift of the energy gap does not seem to affect τ_2 significantly.

Some recent studies failed to detect the $P700^+A_0^-$ state[16,17] under the conditions in which they observed the bleaching of P700 with time constants of 7−18 ps. A_0^- might not have accumulated to a detectable level in these studies, since the time constants for the overall reduction of A_0 could be almost comparable to or longer than the τ_2 determined with MK or PhyQ measured in this study. These results suggest that accumulation of A_0^- can be realized either in PS I preparations with small antenna sizes as used here or under very high excitation density in the ordinary PS I preparations with large antenna sizes (100 Chl/P700) as recently shown by Hastings *et al.*[15,35]

In the purple bacterial RC, one of the two bacteriochlorophylls near P860 functions as the intermediary electron carrier and/or as the superexchange mediator between P860 and BH.[1,36] An X-ray crystallographic study with a 6 Å resolution also suggested the presence of such chlorophylls near P700.[12] Some chlorophyll species might function as the electron mediator between P700 and A_0 or between A_0 and Q_ϕ. However, we were not able to find the absorption changes of the acceptor chlorophyll other than A_0 in this study. The A_0^-/A_0 spectra in Figure 4C, however, show a shoulder at around 670−675 nm. This might indicate the presence of chlorophylls that show an electrochromic shift or redox reactions in a similar time range as the redox reactions of A_0. Such chlorophylls might be located near P700 and A_0.

The τ_2 value of 31 ps for the reaction between A_0^- and MK obtained in the 16 Chl/P700 particles is a little longer than that in the 30 Chl/P700 particles (Table 1). This may reflect the modification of the RC structure by the severe extraction of antenna chlorophylls or may simply reflect some experimental errors. However, the extraction did not seem to significantly change τ_2.

4.3. Comparison of Electron Transfer Kinetics in PS I RC with Those in Purple Bacterial/PS II RCs. The features of electron transfer reactions in PS I are compared with those in PS II and purple bacterial RC complexes (see Figure 5) on the basis of a theoretical framework. In the classical expression of Marcus theory, the rate constant of an electron transfer is written as[37]

$$k_{ET} = \frac{2\pi}{\hbar}\frac{|V(r)|^2}{\sqrt{4\pi\lambda k_B T}}\exp\left[-\frac{(\Delta G^\circ + \lambda)^2}{4\lambda k_B T}\right] \quad (1)$$

where $V(r)$ is the matrix element that couples the electronic wave functions of reactant and product, $-\Delta G^\circ$ is the free energy difference, λ is the reorganization energy of the reaction coordinate, k_B is the Boltzmann's constant, T is the absolute temperature, and $\hbar$ is the Plank's constant. $V(r)$ falls off rapidly with the distance r between donor and acceptor as[37,38]

$$|V(r)|^2 = V(0)^2 \exp(-\beta r) \quad (2)$$

Moser and Dutton[38] assumed that the protein medium acts as a relatively uniform barrier to electron tunneling and proposed an empirical equation with a β value of 1.4 Å^{-1} that relates k_{ET} and the edge-to-edge distance r as

$$\log k_{ET} = 15 - 0.6r - 3.1(\Delta G^\circ + \lambda)/\lambda \quad (3)$$

The energy gap ($-\Delta G^\circ$) between P700*A_0 and $P700^+A_0^-$ states can be estimated to be about 0.25 eV.[8,9] This energy gap is similar to that between P860*BH and $P860^+BH^-$ states (0.2−0.3 eV) in *Rb. sphaeroides* RC[39] (Figure 5). The intrinsic rate constant of the electron transfer from P700* to A_0 is estimated to be equal to or larger than (8 ps)$^{-1}$ if we consider the effect of energy transfer between P700 and other chlorophylls.[31] The intrinsic time constant in PS I RC[31,33−35] may be comparable to that of the corresponding charge separation between P860 and BH in the *Rb. sphaeroides* RC ((3.5 ps)$^{-1}$).[1]

The $-\Delta G^\circ$ between $P700^+A_0^-$ and $P700^+$PhyQ$^-$ (or $P700^+MK^-$) states can be estimated to be around 0.21 eV (Figure 5).[9] This energy gap is smaller than that (0.5−0.7 eV) between $P860^+BH^-$ and $P860^+Q_A^-$ states in the purple bacterial RC.[39] The τ_2 of 23 ps for the reaction between A_0^- and the reconstituted PhyQ or MK is significantly shorter than that (220 ps) between BH^- and Q_A (ubiquinone) in the *Rb. sphaeroides* RC.[39] Studies of electron transfer rates between BH^- and a

variety of reconstituted quinones in the *Rb. sphaeroides* RC showed that λ for this reaction is 0.6 eV and that the time constant for this reaction was never shorter than 220 ps in the range of $-\Delta G°$ between 0.2 and 0.8 eV.[40] Even with the wider variation of $-\Delta G°$, the reaction between BH^- and Q_A does not seem to give a time constant as short as 23 ps.[40]

The molecular geometry of PS I RC thus seems to be responsible for the short τ_2. Equation 3 predicts the edge-to-edge distance between A_0 and PhyQ to be 7.3 Å, if we assume that $-\Delta G°$ and λ cancel each other in the native PS I RC with PhyQ. The calculated distance is shorter than that (9 Å[2,3]) between BH and Q_A by about 2 Å. However, eqs 1 and 2 show that the rate constant does not depend on distance alone even when $-\Delta G° = \lambda$. There might be a difference of the $V(0)$ value between PS I and purple bacterial RCs. The β value may also be affected by the specific protein structure or chlorophyll molecules intervening between the ET components as predicted by the superexchange model.[36,41] Determination of τ_2 with a variety of reconstituted quinones that give wider variation of $-\Delta G°$ is now in progress. The electron transfer rates determined in the primary reactions of PS I RC will increase our understanding of intraprotein electron transfer mechanisms if it is combined with more precise information for the tertiary structure.

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