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ARTICLE *in* BIOCHEMISTRY · JULY 1991

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Articles

Full Replacement of the Function of the Secondary Electron Acceptor Phylloquinone(=Vitamin K_1) by Non-Quinone Carbonyl Compounds in Green Plant Photosystem I Photosynthetic Reaction Centers†

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Received November 14, 1990; Revised Manuscript Received March 6, 1991

ABSTRACT: One-carbonyl quinonoid compounds, fluorenone (fluoren-9-one), anthrone, and their derivatives are introduced into spinach photosystem (PS) I reaction centers in place of the intrinsic secondary electron acceptor phylloquinone (=vitamin K_1). Anthrone and 2-nitrofluorenone fully mediated the electron-transfer reaction between the reduced primary electron acceptor chlorophyll A_0^- and the tertiary electron acceptor iron–sulfur centers. It is concluded that the PS I phylloquinone-binding site has a structure that enables various compounds with different molecular structures to function as the secondary acceptor and that the reactions of incorporated compounds are mainly determined by their redox properties rather than by their molecular structure. Carbonyl groups increase the binding affinity of the quinone/quinonoid compounds but do not seem to be essential to their function. The quinonoid compounds as well as quinones incorporated into the PS I phylloquinone-binding sites are estimated to function at redox potentials more negative than in organic solvents.

In the photosystem I reaction center (PS I RC)[1] of green plants, absorption of light oxidizes the special RC chlorophyll P700 and initiates a series of electron-transfer steps as shown in Figure 1 [see reviews by Andréasson and Vängard (1988), Golbeck (1987), and Mathis (1990)]. The primary electron acceptor A_0 is chlorophyll *a*-690 (Shuvalov et al., 1986; Wasielewski et al., 1987; Mathis et al., 1988), and A_1 is the secondary electron acceptor phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone = vitamin K_1); F_X, F_A, and F_B represents iron–sulfur center X, A, or B (Golbeck, 1987). These cofactors, except F_A/F_B on a 9-kDa small subunit polypeptide, reside on the RC complex made of two 80-kDa molecular polypeptides (Kirsch et al., 1986; Golbeck, 1987).

We developed an extraction/reconstitution method of phylloquinone in spinach PS I particles and provided the direct evidence for the chemical identity of A_1 (Itoh et al., 1987; Itoh & Iwaki, 1988) as confirmed in cyanobacterial membranes (Biggins & Mathis, 1988; Ikegami & Katoh, 1989). One of two phylloquinone molecules contained in the PS I RC complex (Takahashi et al., 1985; Schoeder & Lockau, 1986; Malkin, 1986) is now estimated to function as A_1 (Brettel et al., 1986; Itoh et al., 1987; Ikegami et al., 1987; Biggins & Mathis, 1988; Itoh & Iwaki 1989a), although some arguments still remain (Warden, 1990). The extraction of phylloquinone stops the oxidation of A_0^- and enhances the charge recombination reaction between A_0^- and $P700^+$ (Itoh et al., 1987; Ikegami et al., 1987; Biggins & Mathis, 1988; Itoh & Iwaki, 1989a). This reaction produces a triplet state of P700 ($P700^T$) (Ikegami et al., 1987) or delayed fluorescence (Itoh & Iwaki, 1988) and depresses the $P700^+$ amount detectable in the microsecond–millisecond time range after flash excitation. Reconstitution of phylloquinone (Itoh et al., 1987; Itoh & Iwaki, 1988, 1989a; Biggins & Mathis, 1988) or other quinones (Iwaki & Itoh, 1989, 1990, 1991a; Kim et al., 1989; Biggins, 1990) recovers the rapid oxidation of A_0^- by the quinone and stabilizes $P700^+$ until the microsecond–millisecond time range. The phylloquinone-binding site accepts various benzo-, naphtho-, and anthraquinones (Iwaki & Itoh, 1989, 1990, 1991a,b) as well as quinone site inhibitors (Itoh & Iwaki, 1989b, 1990). We thus proposed to call this electron acceptor phylloquinone (A_1) as Q_ϕ and its binding site as the Q_ϕ site to facilitate comparison with the other quinone functional sites in photosynthetic or respiratory electron-transfer systems (Itoh & Iwaki, 1989b).

† This work is supported by grants-in-aid for Cooperative Research and for Scientific Research from the Japanese Ministry of Education, Science, and Culture to S.I.
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[1] Abbreviations: PS, photosystem, RC, reaction center; A_0 and A_1, PS I primary and secondary (=phylloquinone) electron acceptors, respectively; AN, acetonitrile; DMF, dimethylformamide; E_m, redox midpoint potential; $E_{1/2}$, polarographically measured half-wave redox potential; F_X, F_A, and F_B, iron–sulfur centers X, A, and B; P700, photosystem I primary electron donor chlorophyll *a*; Q_A and Q_B, secondary and tertiary electron acceptor quinones in photosystem II or purple bacterial RF; Q_ϕ, quinones functioning as A_1 (including phylloquinone); $t_{1/2}$, half decay time.

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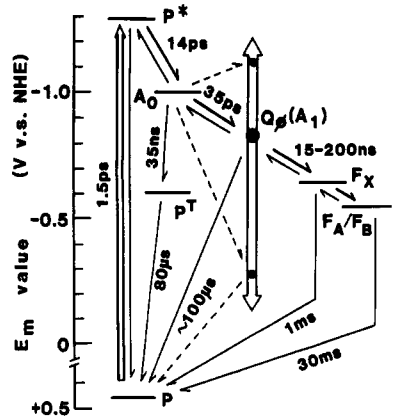

FIGURE 1: Electron-transfer scheme in PS I RC containing various quinones or quinonoids as the secondary acceptor Q_ϕ (A_1) in place of intrinsic phylloquinone. E_m of Q_ϕ is estimated to vary depending on each reconstituted quinone/quinonoid as shown by a vertical blank arrow. Estimated E_m of intrinsic phylloquinone (-0.82 V; Iwaki & Itoh, 1991a) is shown by a filled large circle. Broken lines represent the estimated electron-transfer pathways in the presence of reconstituted quinonoids: P, P700: NHE, normal hydrogen electrode. See text for details.

The ability of quinones to replace the function of intrinsic phylloquinone essentially depended on their redox properties but weakly depended on their molecular structure (Iwaki & Itoh, 1989, 1991a,b). the artificial quinones with appropriate redox potentials rapidly oxidized A_0^- and almost fully reduced the iron–sulfur centers (Iwaki & Itoh, 1989, 1991a). The flexible nature of the Q_ϕ site resembles that of the Q_A site of *Rhodobacter sphaeroides* RC, at which site various artificial quinones (Woodbury et al., 1986; Warncke et al., 1987) or non-quinone compounds (Warncke & Dutton, 1990) replace the function of intrinsic ubiquinone. On the other hand, Biggins et al. (1990) reported that only phylloquinone, but not other quinones like menaquinone or menadione, fully restores the function of the secondary acceptor. They used the resistance of reconstituted quinone to the reduction by added dithionite as the indicator of reconstitution of A_1 (Q_ϕ) since A_1 is expected to show an extremely low E_m and to be resistant to reduction by dithionite. Recently, Biggins (1990) repeated experiments similar to those in Iwaki and Itoh (1989) and obtained a different conclusion. In his study, the effect of methyl viologen, which is assumed to oxidize iron–sulfur centers as the external oxidant, on $P700^+$ kinetics could be seen only when methylnaphthoquinones having long hydrocarbon chains, such as phylloquinone or menaquinone, are reconstituted but not when artificial quinones of small molecular sizes are reconstituted. It was concluded that the PS I phylloquinone-binding site has a strict requirement for the molecular structure of reconstituted quinone to fully regain the function to reduce iron–sulfur centers. This conclusion was again contradictory to ours (Iwaki & Itoh, 1989, 1990, 1991a). However, the reconstitution studies in the two laboratories agree with the point that most of the artificial quinones reconstituted can rapidly oxidize A_0^- and can suppress the charge recombination. The discrepancy resides in whether the reconstituted quinones having different molecular structures can mediate electrons to the iron–sulfur centers. Measurements of turnover of iron–sulfur centers, done recently (Iwaki & Itoh, 1991a), indicated that a wide variety of artificial quinones show this activity. This discrepancy, thus, might have arisen from differences in the experimental conditions.

In this study quinonoid compounds, having only one carbonyl group, were introduced into the PS I RC. The relation between their ability to substitute for intrinsic phylloquinone and their redox properties or molecular structures was investigated. The relation between the molecular structure of quinone/quinonoids and their affinity to the PS I RC is studied in the following paper.

Materials and Methods

Lyophilized PS I particles, obtained by treating spinach chloroplasts with digitonin, were twice extracted with a 1 to 1 mixture of dry and water-saturated diethyl ether, followed by one more extraction with dry diethyl ether according to Itoh et al. (1987); this procedure completely extracted phylloquinone contained at about 2 molecules/P700 in the original PS I particles. The phylloquinone-depleted particles were also depleted of about 90% of the antenna chlorophyll complement and all carotenoids (Itoh et al., 1987). However, P700, A_0, F_X, F_B, and F_A were almost unaffected (Itoh et al., 1987; Ikegami et al., 1987; Ikegami & Itoh, 1987). The extracted particles were dispersed in 50 mM CHES–NaOH buffer, pH 10, and then diluted in 50 mM Tris-HCl buffer, pH 7.5, containing 0.2% (v/v) Triton X-100. After 30 min of incubation, undissolved materials were eliminated by centrifugation. The clear supernatant was about 50× diluted with 50 mM Tris-HCl buffer, pH 7.5, containing 30–60% (v/v) glycerol to give a final P700 concentration of about 0.25 μM and used for the reconstitution and measurements.

To reconstitute quinones or quinonoids into PS I, RC, the suspension of the phylloquinone-extracted PS I particles was incubated overnight at 0 °C in the dark with varied concentrations of quinone/quinonoids dissolved in dimethyl sulfoxide as described previously (Iwaki & Itoh, 1989). A couple of ascorbate and dichloroindophenol (at final concentrations of 10 mM and 0.1 mM, respectively) was added before the measurement to fully reduce $P700^+$. Chemicals used for the reconstitution were anthrone (Wako, Osaka, and a gift from Dr. Y. Sakata of Osaka University), fluorenone (=fluoren-9-one, Wako), 2-nitrofluorenone, 2-fluorofluorenone, 2,4,5,7-tetranitrofluorenone, and 2-aminofluorenone (Aldrich, Milwaukee, WI), benzanthrone, 2,7-dinitrofluorenone, 2,4,7-trinitrofluorenone (Tokyokasei, Tokyo), and quinones as described previously (Iwaki & Itoh, 1989). The reconstituted PS I particles were assayed by measuring the absorption change induced by a flash (532 nm, 10 ns at 0.5–1 Hz, 0.4 mJ) from a Nd-YAG laser (DCR2-10, Quanta Ray) in a split-beam spectrophotometer at 6 °C as described previously (Iwaki & Itoh, 1989) or at low temperatures in an Oxford cryostat (DN704). Signals were averaged between 16 and 128 scans as required in each case.

Results

Flash-Induced Absorption Change of P700 in Quinonoid-Containing PS I Particles. In the ether-extracted PS I particles in which intrinsic phylloquinone is extracted, only a small amount of $P700^+$ was detected in the microsecond–millisecond time range after excitation with a laser flash (Figure 2a). This is due to the rapid return of an electron on A_0^- to $P700^+$ (charge recombination) with a characteristic $t_{1/2}$ of 35 ns as shown in Figure 1 (Ikegami et al., 1987; Itoh & Iwaki, 1988; Mathis et al., 1988). This reaction produces either the delayed fluorescence (Itoh & Iwaki, 1988) or the triplet state of P700 ($P700^T$), which decays with a $t_{1/2}$ of 80 μs and also is detectable at 430 nm. The $t_{1/2}$ of $P700^T$ decay is longer than that in the original PS I particles since the carotenoids, which quench $P700^T$, are fully extracted in this preparation. The flash excitation also induced a small amount of $P700^+$, which was slowly reduced by an added ascorbate–dichloroindophenol

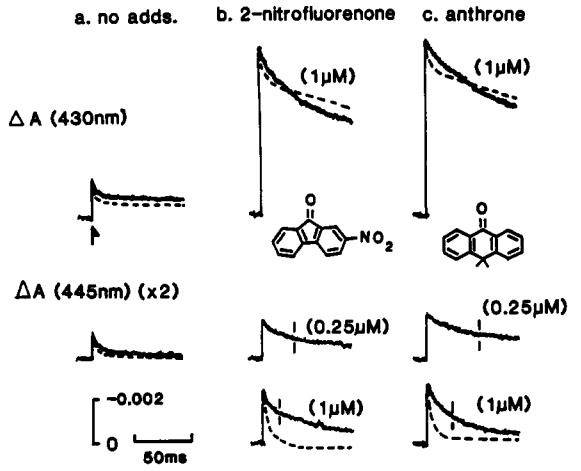

FIGURE 2: Flash-induced difference absorption kinetics at 430 and 445 nm in the ether-extracted PS I particles containing 2-nitrofluorenone (b) and anthrone (c) in place of intrinsic phylloquinone. Broken lines represent kinetics observed on further addition of 30 μM benzyl viologen in each case. The reaction mixture contained PS I particles equivalent to 0.29 μM P700, 20% (v/v) glycerol, and 50 mM Tris-HCl buffer, pH 7.5. Concentrations of 2-nitrofluorenone and anthrone added are shown in brackets; the temperature was 6 °C. Other experimental conditions are described in Materials and Methods.

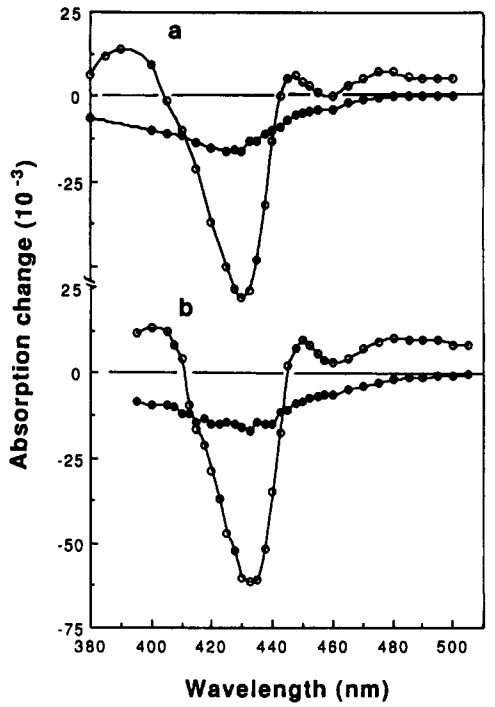

FIGURE 3: Difference spectra of the flash-induced absorption change in the ether-extracted PS I particles containing 2-nitrofluorenone (a) and anthrone (b) in the presence of benzyl viologen. Concentrations of 2-nitrofluorenone, anthrone, and benzyl viologen were 10, 10, and 30 μM, respectively. ○ and ● represent the absorption spectra of the slow (with a $t_{1/2}$ longer than 100 ms) and the fast decay components (with a $t_{1/2}$ of 3 ms), respectively. Other conditions were simlar to those in Figure 2.

couple with a $t_{1/2}$ longer than 100 ms.

Reconstitution with 2-nitrofluorenone increased the $P700^+$ extent in the microsecond–millisecond time range (Figure 2b) followed by a slow decay, indicating that this compound suppresses the charge recombination in place of intrinsic phylloquinone by rapidly oxidizing A_0^-. The high initial extent indicates that the oxidation of A_0^- by this compound proceeds much faster than the charge recombination, as reported with methylnaphthoquinone ($t_{1/2}$ = 150 ps) by Kim et al. (1990). The slow following decay of $P700^+$ resembles those reported in the case of reconstitution of phylloquinone or other quinones (Iwaki & Itoh, 1989). Anthrone showed almost the same effect as 2-nitrofluorenone. Similar effects were also seen with 2-fluorofluorenone (see Figure 4) or benzanthrone (see Figure 7). We, therefore, concluded that these quinonoid compounds function as the electron acceptor to A_0^- in place of phylloquinone and suppress the charge recombination. When benzyl viologen was further added, the initial decay at 430 nm was accelerated while the following decay was decelerated (broken lines).

At the isosbestic wavelength (445 nm) of the $P700^+/P700$ couple (Figure 2, lower traces), 2-nitrofluorenone or anthrone suppressed the $P700^T$ rapid decay and elevated the extent of slow decay. The decay rate was similar to that of the initial fast decay at 430 nm. Further addition of benzyl viologen significantly accelerated the decay rate at 445 nm. In normal PS I particles, benzyl viologen oxidizes $(F_A/F_B)^-$ as an external electron acceptor and decelerates the reduction of $P700^+$ by suppressing the reduction by $(F_A/F_B)^-$ (Hiyama & Ke, 1972; Golbeck, 1987). The accelerated fast decay phases observed at 445 and 430 nm are thus considered to reflect $(F_A/F_B)^-$ oxidation by benzyl viologen as confirmed by the difference spectrum (see Figure 3).

These results indicate that 2-nitrofluorenone and anthrone fully mediate the reduction of F_A/F_B in replace of phylloquinone (Q_ϕ) as previously reported in the reconstitution of quinones (Iwaki & Itoh, 1989, 1990, 1991a). It is also noted that even without benzyl viologen the decay rate at 445 nm became faster at the higher concentrations of anthrone or 2-nitrofluorenone (e.g., at 1 μM in Figure 2). This shows that these quinonoid compounds also function as the external electron acceptor to $(F_A/F_B)^-$ as benzyl viologen does. Therefore, they, especially at high concentrations, decrease the apparent effectiveness of benzyl viologen. The quinonoids seemed to oxidize $(F_A/F_B)^-$ at the site other than the Q_ϕ site since the acceleration of the decay rate was more prominent at the higher quinonoid concentrations at which the initial extent of the flash-induced absorption change was almost saturated (not shown). This type of effect was often observed with quinones of small molecular size but was weak in the case of phylloquinone or menaquinone, which shows high affinity to the Q_ϕ site (Iwaki & Itoh, 1991) and low solubility in water. This can be seen from the faster decay rate (mainly with a $t_{1/2}$ of 30 ms) of the slow phase when phylloquinone was reconstituted (see Figure 4). This may explain the results of Biggins (1990), who detected the effect of methyl viologen only in the PS I RC reconstituted with the high-affinity quinones having long hydrocarbon chains but not in the RC reconstituted with the small and low-affinity quinones at relatively high concentrations (25 μM). The effect of methyl viologen might have been difficult to detect by the slow-response experimental setup (DC-3-kHz bandwidth) used by Biggins (1990) since the decay rate of F_A/F_B under these conditions is already fast before the addition of methyl viologen.

The fast and slow phases observed in the presence of benzyl viologen gave the difference spectra shown in Figure 3. The spectrum of the fast phase is ascribed to that of F_A/F_B (P430) (Hiyama & Ke, 1972), showing a broad absorption band peaking at 430 nm, and the slow phase of $P700^+/P700$. These spectra confirm that 2-nitrofluorenone and anthrone can reduce F_A/F_B presumably via F_X. These activities of quinonoid compounds were stable at temperatures lower than 10 °C but were rapidly lost above 20 °C as the RC protein denatures, as reported by Ikegami and Katoh (1989), indicating that the

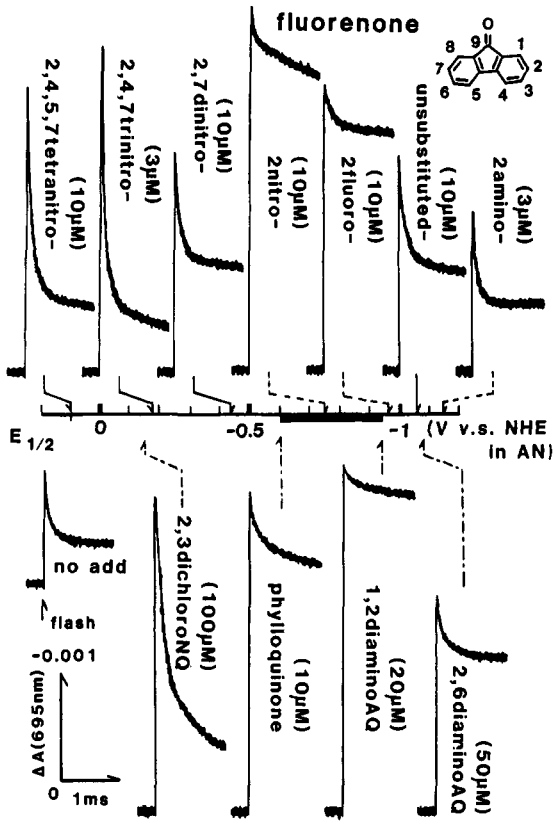

FIGURE 4: Flash-induced absorption kinetics of P700 in the ether-extracted PS I particles reconstituted with fluorenone derivatives (upper traces) and quinones (lower traces). Experimental conditions were similar to those in Figure 1. $E_{1/2}$ values in AN of fluorenones, indicated by solid lines on the horizontal axis, are obtained from Kuder et al. (1978), and that of 2-nitrofluorenone was estimated by interpolation by use of the $E_{1/2}$ values of other nitrofluorenone derivatives. $E_{1/2}$ values of 2-fluoro- and 2-aminofluorenones, which have not been measured yet, are represented at the positions 0.1 V more positive or negative than the $E_{1/2}$ of unsubstituted fluorenone, respectively, for convenience of comparison. These two $E_{1/2}$ values, therefore, have no experimental basis. $E_{1/2}$ values of quinones in AN were estimated by assuming the negative shift of 0.1 V from those measured in DMF in each case as described in text. Other experimental conditions were similar to those in Figure 2. $E_{1/2}$ values are expressed with respect to normal hydrogen electrode (NHE).

quinonoid reactions are not the artificial ones with bulk chlorophylls.

Redox Potentials of Fluorenone Compounds and Their Effectiveness in Recovering Electron Transfer in PSI RC. Each addition of a nitro group to fluorenone shifts the half-wave redox potential ($E_{1/2}$) measured electrochemically in organic solvents by about 0.3 V to the positive direction (Kuder et al., 1978). On the other hand, addition of amino or alkyl groups are expected to shift $E_{1/2}$ to the negative direction and halogens to the positive direction. We can, thus, cover a wide redox range with derivatives of fluorenone. In addition to 2-nitrofluorenone used in Figure 2, tetranitro-, trinitro-, dinitro-, 2-fluoro-, and aminofluorenones and unsubstituted fluorenone were introduced into PS I RC in place of phylloquinone (Figure 4). $P700^+$ kinetics with fluorenone derivatives are displayed in the order of their $E_{1/2}$ values (some of the $E_{1/2}$ values shown by broken lines are roughly estimated as described in the figure legends) in acetonitrile (AN). Concentrations of each compound were adjusted to give the optimal effect. A high extent of $P700^+$ was observed with 2-nitrofluorenone and 2-fluorofluorenone, followed by a slow decay as seen in Figure 1. Tetra- and trinitrofluorenones also induced the high initial extent, followed by a fast decay with a $t_{1/2}$ of about 60 μs. Unsubstituted fluorenone and amino- and dinitrofluorenones, on the other hand, partially elevated the slow decay.

In the quinone-reconstitution studies done previously (Iwaki & Itoh, 1989, 1990, 1991a), we used $E_{1/2}$ values measured in the organic solvent dimethylformamide (DMF) to represent the redox properties of quinones. These values were measured by P. L. Dutton's group and were used in their study of quinone reconstitution into the Q_A site of *Rb. sphaeroides* RC (Dutton et al., 1982; Woodbury et al., 1986). We, however, have not found $E_{1/2}$ in DMF of some fluorenones but found their $E_{1/2}$ measured in AN (Kuder et al., 1978). The $E_{1/2}$ in DMF of unsubstituted fluorenone was measured to be 0.08 V more positive ($E_{1/2}$ = −0.98 V vs NHE; P. L. Dutton and M. R. Gunner, personal communication) and that of trinitrofluorenone to be 0.15 V more positive ($E_{1/2}$ = −0.03 V; Kuder et al., 1978) than those measured in AN, respectively. Thus, in Figure 4, $E_{1/2}$ values of quinones are indicated to be 0.1 V more negative than those in DMF, assuming that quinones show similar shifts of $E_{1/2}$ upon the change of solvent.

The kinetics of $P700^+$ in the PS I RC reconstituted with characteristic quinones, reported previously (Iwaki & Itoh, 1989), are also shown in Figure 4. Only the quinones with $E_{1/2}$ values between −0.5 and −0.85 V in DMF (presumably between −0.6 and −0.95 V in AN as shown by the shaded zone) were shown to fully reduce F_A/F_B as represented by the kinetics with phylloquinone or 1,2-diaminoanthraquinone (Figure 4). The quinones with a more positive $E_{1/2}$ reduce $P700^+$ more rapidly with a $t_{1/2}$ of 110 μs as seen with 2,3-dichloronaphthoquinone (Figure 4). The quinones with a more negative $E_{1/2}$, on the other hand, only partially recover the slow $P700^+$ decay as seen with 2,6-diaminoanthraquinone. From these results, a previous study concluded that most of quinones exhibit an E_m in situ at the PS I Q_ϕ site about 0.3 V more negative than their $E_{1/2}$ in DMF (Iwaki & Itoh, 1989, 1990, 1991a).

The $E_{1/2}$ of 2-nitrofluorenone in AN is estimated to be around −0.75 V and to be intermediate between that of phylloquinone and 1,2-diaminoanthraquinone (Kuder et al., 1978). This compound fully increased the $P700^+$ slow decay phase as shown in Figures 2 and 3. The E_m in situ at the Q_ϕ site of this compound is, therefore, assumed to be negative enough to reduce F_X, whose E_m is around −0.7 V (Ke et al., 1977), i.e., to be more negative than −0.8 V. Tri- and tetranitrofluorenones show higher $E_{1/2}$ values in AN than dichloronaphthoquinone and induced similar kinetics to those seen with dichloronaphthoquinone, although the $P700^+$ reduction rates were about twice faster than that observed with the latter. Neither the absorption change at 445 nm nor the effects of benzyl viologen were detected with these high-potential fluorenones (see Figure 5), indicating that they did not reduce the iron–sulfur centers but directly reduced $P700^+$. Smaller enhancement of the flash-induced initial extent of $P700^+$ by unsubstituted fluorenone and aminofluorenone may be due to their negative $E_{1/2}$ values, which make them difficult to be fully reduced by A_0^-, as previously assumed in the case of the low-potential 2,6-diaminoanthraquinone (Iwaki & Itoh, 1989). The results with fluorenone derivatives suggest that their E_m values in situ at the PS I Q_ϕ site are related to their $E_{1/2}$ values in organic solvent and that the shift of E_m with respect to $E_{1/2}$ in AN is about −0.1 to −0.3 V, in a range similar to that estimated for quinones. The inefficiency of dinitrofluorenone remains to be studied.

Exact $E_{1/2}$ values of anthrone and benzanthrone are not found in the literature and are assumed to be more negative than anthraquinone. The results in the present study suggest

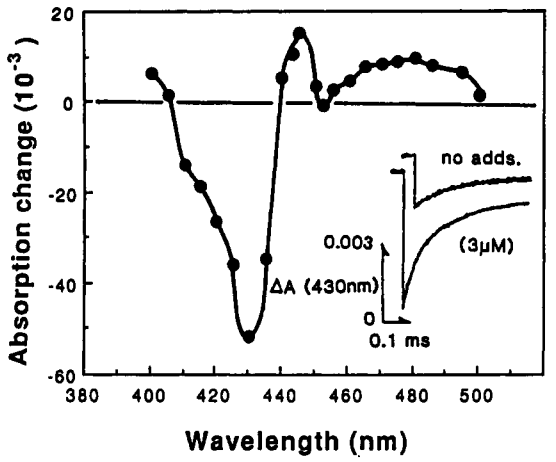

FIGURE 5: Difference absorption spectrum of the ether-extracted PS I particles reconstituted with 2,4,7-trinitrofluorenone at 77 K. The extent of flash-induced absorption change at 1 μs after the flash excitation, as shown in Figure 4, is plotted. The insert traces represent the flash-induced absorption kinetics at 77 K in the presence and absence of trinitrofluorenone. Experimental conditions were similar to those in Figure 2 except that the measurements were done at 77 K in a cryostat in the presence of 50% glycerol.

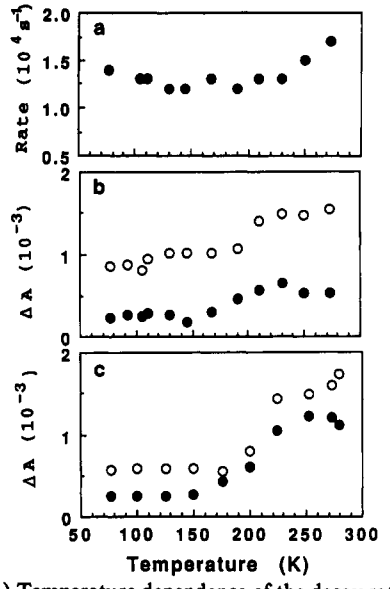

FIGURE 6: (a) Temperature dependence of the decay rate of the initial fast phase measured at 430 nm in the PS I RC reconstituted with 2,4,7-trinitrofluorenone. (b, c) Temperature dependence of the extent of flash-induced absorption changes in the ether-extracted PS I particles reconstituted with 2,4,7-trinitrofluorenone (b) and 2-nitrofluorenone (c). ○ and ● represent the extent of $P700^+$ at 1 μs and 0.8 ms, respectively, after the flash excitation. Concentrations of trinitrofluorenone and 2-nitrofluorenone were 3 and 10 μM, respectively. Other experimental conditions were similar to those in Figure 5.

that the E_m of anthrone in situ at the Q_ϕ site is not very different from that of anthraquinones or 2-nitrofluorenone. The E_m of benzanthrone might be more negative since this compound gave $P700^+$ kinetics intermediate between those with 2-fluorofluorenone and unsubstituted fluorenone (not shown).

Reaction of P700 at Low Temperatures in the PS I RC Complex Containing Nitrofluorenones. If the derivatives of fluorenones and anthrones actually function at the Q_ϕ site then they are expected to work at cryogenic temperatures as does intrinsic phylloquinone. Additions of fluorenones increased the amount of flash-induced $P700^+$ also at low temperatures (Figures 5 and 6). The difference absorption spectrum in the presence of trinitrofluorenone at 77 K is ascribed to

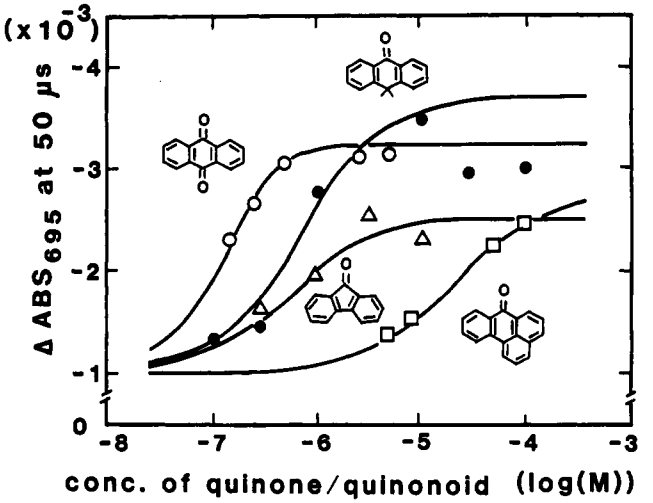

FIGURE 7: Effects of concentrations of anthraquinone and quinonoids on the extent of flash-induced $P700^+$. Initial extent of flash induced $P700^+$ was measured as in Figure 4. Symbols ○, ●, □, and △ represent 9,10-anthraquinone, anthrone, benzanthrone, and fluorenone, respectively.

Table I: Dissociation Constant K_d of Quinones and Quinonoid Compounds at the Q_ϕ Site in PS I RC Complex

compounds	log K_d (M)
quinones	
phylloquinone	<−10
9,10-anthraquinone	−7.7
anthrones	
anthrone	−6.3
benzanthrone	−4.7
fluorenones	
unsubstituted	−6.5
2-nitro-	−6.5
2,7-dinitro-	−6.1
2,4,7-trinitro-	−6.5
2,4,5,7-tetranitro-	−6.2

$P700^+$/P700 (Figure 5) and is clearly different from that of $P700^T$/P700, which is often seen in damaged PS I preparations. Similar results were also observed with tetranitrofluorenone (not shown). The rapid 60-μs decay phase seen in the trinitrofluorenone-reconstituted PS I RC decreased slightly on cooling to 230 K and was almost temperature independent until 77 K, although the extent of small slow phase decreased below 200 K (Figure 6a,b). These features are similar to those observed with high-potential quinones (unpublished data) and suggest that the reaction between tri- or tetranitrofluorenone radical anions and $P700^+$ is an apparently activationless reaction between 230 and 77 K. On the other hand, the extent of flash-induced $P700^+$, especially of the slow phase, was decreased at temperatures below 200 K in the 2-nitrofluorenone-containing RC, (Figure 6c). This is explained by the accumulations of $(F_A/F_B)^-$ and $P700^+$ under repetitive flash excitation since the reduction of $P700^+$ by $(F_A/F_B)^-$ stops at this temperature [see Golbeck (1987)].

Affinity of Quinonoid Compounds for the PS I Phylloquinone Binding Q_ϕ site. The initial extent of flash-induced $P700^+$ as shown in Figure 4 is assumed to be proportional to the amount of PS I RC containing reconstituted quinonoids. Dependence of the extent on the concentration of quinonoid compound was measured to calculate their dissociation constant K_d at the Q_ϕ site (Figure 7). K_d values obtained in this way are listed in Table I. K_d values of unsubstituted fluorenone or anthrone were about an order higher than that of anthraquinone, suggesting that the second carbonyl group of anthraquinone contributes to its tighter binding. The higher

K_d value of benzanthrone further suggests that the larger molecular size decreases the affinity. Additions of nitro groups are expected to make fluorenone less hydrophobic and to give a higher K_d value. However, the K_d value was only slightly increased by the addition of nitro groups.

Discussion

Non-quinone aromatic compounds 2-nitrofluorenone and anthrone almost fully replaced the function of phylloquinone in PS I RC. This indicates the rather nonspecific nature of phylloquinone-binding Q_ϕ site for accepting quinone/quinonoid molecules and indicates that the structural features of intrinsic phylloquinone, two carbonyls, a phytyl chain, and the high hydrophobicity, are not essential in its activity as the secondary electron acceptor, although they contribute to its extremely tight binding to the Q_ϕ site.

The function of fluorenone and anthrone derivatives at the Q_ϕ site changes depending on the redox properties. E_m values of these compounds at the Q_ϕ site can be estimated to be 0.1–3.0 V more negative than their $E_{1/2}$ values in AN (or in DMF). The shift of E_m is in a range similar to that (−0.3 V) observed when quinones are reconstituted to the Q_ϕ site (Iwaki & Itoh, 1991a). More quantitative studies may be called for, since the E_m values of quinones in situ at the Q_A site of *Rb. sphaeroides* RC are reported to be only roughly related to their $E_{1/2}$ in DMF (Woodbury et al., 1986). The PS I Q_ϕ site is concluded to shift the E_m of quinones or one-carbonyl compounds to the negative direction. This is, however, opposite to the situation in the Q_A sites of purple bacterial and plant PS II RCs, where quinones show E_m of 0.2–0.4 V more positive than their $E_{1/2}$ in DMF (Woodbury et al., 1986). The Q_ϕ site, thus, seems to have a unique structure in its quinone-binding niche. The PS I RC produces stronger reducing power than PS II or purple bacterial RCs so that the compounds with extremely negative $E_{1/2}$ values, such as anthrone, aminofluorenone, unsubstituted fluorenone, or 2,6-diaminoanthraquinone, some of which were unable to be reduced at the Q_A site (Woodbury et al., 1986), can be reduced and fully function as the Q_ϕ. The PS I Q_ϕ site, thus, may have a wider choice of compounds in the reconstitution studies.

The lower affinities of quinonoid compounds may indicate that one of the quinone carbonyls forms a hydrogen bond with amino acid residues in the Q_ϕ site and to about 2–3 kcal/mol (calculated from the shift of log K_d) increase of the binding free energy. The contribution of carbonyl to the binding may be more significant if the higher hydrophobicity of anthrone is taken into account since the quinones of higher hydrophobicity are known to show the tighter binding at the Q_ϕ site (Iwaki & Itoh, 1989, 1991b).

Hydrogen bonding of quinone carbonyl is expected to stabilize semiquinone and to contribute to shift E_m to the positive direction. However, the shifts of E_m of fluorenone derivatives from their $E_{1/2}$ in organic solvent are estimated to be similar to those of quinones. The loss of carbonyl, therefore, dose not seem to significantly affect the shift of E_m of quinone/quinonoid compounds at the Q_ϕ site, although it decreased the affinity to the Q_ϕ site. The negative shift of E_m values of quinones/quinonoids at the Q_ϕ site, therefore, may mainly be due to other features of the quinone-binding niche that destabilize the semiquinone radical, such as the existence of a negative charge or the electronic interaction between π orbitals of quinone and the nearby aromatic amino acid side chain. Quantitative studies with the wider variety of compounds, now undergoing, may answer this.

Small effects of nitro groups on the binding affinity of fluorenone derivatives still remain to be interpreted. Additions of nitro groups are expected to increase the steric hindrance since only a space as large as the anthraquinone ring size has been assumed to be freely occupied by the reconstituted quinones (Iwaki & Itoh, 1991b), as shown by the low affinity of benzanthrone. The nitro groups of fluorenone may specifically interact with some amino acid residues in the binding niche.

The reduction rate of $P700^+$ in the PS I RC reconstituted with tri- or tetranitrofluorenone was almost temperature-independent. This strongly suggests that the fluorenones are bound to the specific site in the PS I RC at a close distance from A_0. Although the reason for the faster reaction rate of $P700^+$ with fluorenone ($t_{1/2}$ = 60 μs) than with the high-potential quinone ($t_{1/2}$ = 110 μs) is not yet clear, it is clear that the non-quinone compounds can properly reconstitute efficient electron-transfer reaction in PS I RC. A temperature independence of the rate was also reported in the reaction between the oxidized electron-donor bacteriochlorophyll dimer cation and the $Q_A^{\bullet-}$ of reconstituted quinones in purple bacterial RCs (Gunner & Dutton, 1989). The activationless feature of the electron-transfer reaction in photosynthetic RCs seems to be one of the fundamental features of intraprotein electron transfer in which various frequency modes of reorganization of reaction coordinates are coupled to the electron transfer (Gunner & Dutton, 1989).

The Q_ϕ site in the PS I RC, as well as the Q_A sites of purple bacterial RC (Woodbury et al., 1986; Gunner & Dutton, 1989; Warncke et al., 1990), enables various artificial quinone/quinonoid compounds to function as the secondary acceptor. This contradicts the view proposed by Biggins (1990) that the PS I RC differs from the purple bacterial RCs in that some molecular structure specific for intrinsic phylloquinone is indispensable to fully recover the Q_ϕ function. This idea now seems to lose its experimental basis, although the discrepancy might come from the different extraction/reconstitution procedures. The replacement of the cofactor quinone initiated in *Rb. sphaeroides* RCs (Okamura et al., 1975; Dutton et al., 1982; Gunner et al., 1982) and in PS I RC (Itoh et al., 1987; Biggins & Mathis, 1988) creates a new photochemical energy-conversion system and will contribute to a deeper understanding of the electron-transfer mechanism.

Acknowledgments

We thank Drs. P. L. Dutton and K. Warncke of The University of Pennsylvania for their stimulating discussions and for allowing us to use $E_{1/2}$ values from their unpublished manuscript, Dr. Y. Sakata of Osaka University for his kind gift of anthrone, and Dr. J. H. Golbeck of University of Nebraska-Lincoln for his kind review of the manuscript.

Registry No. Phylloquinone, 84-80-0; 9,10-anthraquinone, 84-65-1; anthrone, 90-44-8; benzanthrone, 82-05-3; fluorenone, 486-25-9; 2-nitrofluorenone, 3096-52-4; 2,7-dinitrofluorenone, 31551-45-8; 2,4,7-trinitrofluorenone, 129-79-3; 2,4,5,7-tetranitrofluorenone, 746-53-2; 2-fluorofluorene, 343-01-1; 2-aminofluorene, 3096-57-9; 2,3-dichloronaphthoquinone, 117-80-6; 1,2-diaminoanthraquinone, 1758-68-5; 2,6-diaminoanthraquinone, 131-14-6.

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