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# Structure of the Phylloquinone-Binding ( $Q_\phi$ ) Site in Green Plant Photosystem I Reaction Centers: The Affinity of Quinones and Quinonoid Compounds for the $Q_\phi$ Site<sup>†</sup>

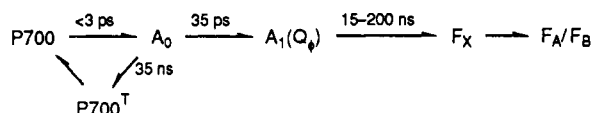
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**ABSTRACT:** The dissociation constants ( $K_d$ ) between the phylloquinone-binding site (designated as the  $Q_\phi$  site) and 23 quinones and 2 quinonoid compounds were measured in spinach photosystem I reaction centers.  $K_d$  values were calculated from the dependency of the recovery of the flash-induced stable oxidation of the primary donor chlorophyll P700 in the phylloquinone-extracted reaction center on the concentration of added compounds. The binding free energy, calculated from the  $K_d$  value of quinones with nonpolar substituted groups, linearly depended on their partition coefficients between water and cyclohexane, but only if their molecular sizes are smaller than anthraquinone. The quinones with larger molecular sizes showed a lower affinity than expected from their hydrophobicities. This suggests that the quinone-binding domain is hydrophobic and that its size is similar to that of anthraquinone. The interaction other than the hydrophobic one was also estimated to stabilize the binding by  $-5.7$  kcal/mol for alkylated quinones. Deletion of one of the carbonyls of *p*-quinones significantly decreased the binding affinity. This suggests a hydrogen bond or a  $\pi$ - $\pi$  electronic interaction between quinone and the  $Q_\phi$  site. Effects of halogens and amino substitutions on the binding affinity were also studied. The structure of the quinone-binding site in the photosystem I reaction center is deduced from these results.

In the photosystem I reaction center (PS I RC)<sup>1</sup> of the green plant photosynthetic system, absorption of light induces charge separation between the donor chlorophyll *a* (P700) and the primary acceptor chlorophyll *a* ( $A_0$ ), and an electron on  $A_0^-$  is transferred to the secondary acceptor ( $A_1$ ) and then to the iron-sulfur centers  $F_X$  and  $F_A/F_B$  as shown below [see reviews by Golbeck (1987) and Lagoutte and Mathis (1989)].



The PS I RC complex binds two molecules of phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone = vitamin  $K_1$ ) and one of them functions as  $A_1$  (Takahashi et al., 1985; Malkin, 1986; Schoeder & Lockau, 1986) presumably at an extremely negative potential ( $E_m \approx -820$  mV; Iwaki & Itoh, 1991). The function of the other molecule of phylloquinone is not yet clear.

We have shown that the phylloquinone can be completely extracted by diethyl ether from spinach PS I RC (Itoh et al., 1987) as later confirmed in cyanobacterial membranes (Biggins & Mathis, 1988; Ikegami & Katoh, 1989). The phylloquinone-binding site accepts a variety of artificial quinones, which can replace the function of phylloquinone (Iwaki & Itoh, 1989; Itoh & Iwaki, 1991). Quinone site inhibitors such as *o*-phenanthroline, myxothiazol, and antimycin A competitively inhibit the reconstitution of quinone (Itoh & Iwaki, 1989b). This is well interpreted by the dissociation equilibrium between the quinone or the inhibitor and the site. We here denote the reconstituted quinone or originally bound phylloquinone, which functions as  $A_1$ , to be  $Q_\phi$  and denote its binding site the  $Q_\phi$

site as proposed previously (Itoh & Iwaki, 1989b).

The function of  $Q_\phi$  as the electron mediator between  $A_0^-$  and the iron-sulfur centers, mainly depends on the redox midpoint potential ( $E_m$ ) of its semiquinone radical/quinone couple but does not critically depend on the structure of the quinone (Iwaki & Itoh, 1989). Even when non-quinone compounds, such as fluorenone or anthrone, are reconstituted into the PS I RC, they can fully mediate the electron transfer between  $A_0$  and the iron-sulfur centers (Itoh & Iwaki, 1991). This is somewhat different from the recent report of Biggins (1990), who concluded that only naphthoquinones with long hydrocarbon tails, but not the tailless ones, can mediate the reduction of iron-sulfur centers, although the latter functioned to rapidly oxidize  $A_0^-$ . The discrepancy may arise from the differences in the measurement systems since we detected almost full reduction of the iron-sulfur centers under similar conditions (Iwaki & Itoh, 1991), or it may reflect differences in the quinone-extraction/reconstitution procedures.

The  $Q_\phi$  phylloquinone is tightly bound to the site and undergoes a one-electron reaction without leaving the site similar to the case of the  $Q_A$  quinones of purple bacterial (ubiquinone or menaquinone) and PS II (plastoquinone) RCs. RCs of green sulfur bacteria seem to have menaquinones that function in a way similar to  $Q_\phi$  (Brok et al., 1986; Nitschke et al., 1987). On the other hand, in the  $Q_B$  sites of RCs of purple bacteria and PS II, quinones seem to be released from the binding sites when they are doubly reduced and protonated. The difference in the quinone functions seems to arise from

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<sup>1</sup> Abbreviations:  $A_0$ , photosystem I primary acceptor;  $A_1$ , photosystem I secondary acceptor;  $F_X$ ,  $F_A$ , and  $F_B$ , iron-sulfur centers X, A, and B in photosystem I; *P*, partition coefficient; P700, photosystem I primary donor; PS, photosystem;  $Q$ , quinone;  $Q_A$  and  $Q_B$ , secondary and tertiary electron acceptor quinones in photosystem II and purple bacterial reaction center; RC, reaction center;  $Q_\phi$ , quinones functioning as  $A_1$  (including phylloquinone).

different features of the protein environment in the quinone-binding niche, which is different from that in a statically homogeneous organic solvent. However, the structure of the  $Q_\phi$  site is not yet known, although the tertiary structures of the  $Q_A$  site in *Rhodospseudomonas viridis* and *Rhodobacter sphaeroides* RCs were shown by X-ray crystallography (Michel et al., 1986; Allen et al., 1987).

In this study, structure of the  $Q_\phi$  binding niche in the PS I RC complex is estimated by studying the relationship between the structures of quinone/quinonoid compounds and their affinity for the  $Q_\phi$  site in the quinone-extracted PS I RC.

**Theoretical Consideration of the Quinone Affinity to the Reaction Center.** The free energy change when a quinone (Q) moves from the aqueous phase to its binding site in PS I RC is expressed as

$$Q \cdot RC \xrightleftharpoons{K_d} Q + RC \quad (1)$$

$$K_d = \frac{[Q][RC]}{[Q \cdot RC]} \quad (2)$$

$$-\Delta G_t = -RT \ln K_d^{-1} \quad (3)$$

where  $K_d$  is a dissociation constant,  $\Delta G_t$  is the apparent total change in Gibbs's free energy,  $R$  is the gas constant, and  $T$  is the absolute temperature. The free energy change is assumed to involve the contributions of nonpolar (hydrophobic) and specific interactions between quinone and the binding site. The binding of quinone at the  $Q_\phi$  site, thus, is imaginarily divided into two sequential steps; the first is the replacement of a quinone from the aqueous solution into the hydrophobic domain of the protein, and the second is the stabilization of quinone by additional specific interactions with some amino acid residues in the binding niche inside the RC protein. The free energy changes in these two steps are expressed by  $\Delta G_{w-h}$  and  $\Delta G_{sp}$ , respectively. The term  $\Delta G_t$  is then represented as

$$\Delta G_t = \Delta G_{w-h} + \Delta G_{sp} \quad (4)$$

$\Delta G_{w-h}$  may be approximated by the free energy change  $\Delta G_{w-c}$  of moving a quinone from water into a nonpolar solvent, cyclohexane, which may provide an environment similar to the hydrophobic interior of the RC protein.

$$\Delta G_t \approx \Delta G_{w-c} + \Delta G_{sp} \quad (5)$$

The term  $\Delta G_{w-c}$  is related to the partition coefficient  $P$  of the quinone between water (w) and cyclohexane (c) phases:

$$[Q]_w \xrightleftharpoons{P} [Q]_c \quad (6)$$

$$P = \frac{[Q]_c}{[Q]_w} \quad (7)$$

$$\Delta G_{w-c} = -RT \ln P \quad (8)$$

Substitution in eq 5 by eq 8 leads to

$$\Delta G_t \approx -RT \ln P + \Delta G_{sp} \quad (9)$$

$P$  values for cyclohexane/water of quinones or quinonoid compounds were measured and provided by P. L. Dutton and M. R. Gunner (personal communication). This type of analysis, initiated by Dutton et al. (Dutton et al., 1982; Gunner et al., 1982) in the  $Q_A$  site of *Rb. sphaeroides* RC, has shown the effectiveness of the use of the partition coefficient (Gunner et al., 1985; Warncke et al., 1987; Warncke & Dutton, 1990). We here apply this type of analysis to the  $Q_\phi$  site and estimate the specific interaction between each compound and the  $Q_\phi$  site to map out the structure of the  $Q_\phi$  site.

## MATERIALS AND METHODS

**Preparation of Quinone-Substituted PS I Particles.** Lyophilized PS I particles (about 20–40 mg) obtained by treating spinach chloroplasts with digitonin were extracted two times with 35 mL of 50% water-saturated diethyl ether, which is a 1 to 1 mixture of diethyl ether dried by  $Na_2SO_4$ , and that containing water at the saturation level. The particles were once extracted with dry diethyl ether to extract residual phyloquinone completely without further damage to the other components. This treatment extracted all the phyloquinone, about 90% of the antenna chlorophylls, and all carotenoids. P700,  $A_0$ ,  $F_X$ ,  $F_A$ , and  $F_B$  are almost unaffected by the treatment (Itoh et al., 1987).

The extracted particles were dispersed in 0.5 mL of 50 mM CHES buffer, pH 10.0, and then diluted to 2 volumes with 50 mM Tris buffer, pH 7.5, followed by addition of Triton X-100 to give a final concentration of 0.2%. After 30 min of incubation in the dark, undissolved materials were eliminated by a centrifugation at 10000g for 3 min. The clear green supernatant of phyloquinone-extracted PS I particles was diluted to 50–100 mL in 50 mM Tris buffer, pH 7.5, containing 20% glycerol. The suspension was then divided into 25–50 plastic cuvettes to give 2 mL of suspensions containing about 0.25  $\mu$ M PS I RC concentration. To each cuvette was then added 1–40  $\mu$ L of an ethanol or dimethyl sulfoxide solution of varied amounts of quinone or quinoid compounds, and the solution was mixed rigorously with a Vortex mixer and incubated for 24 h in the dark at 0 °C to accomplish the reconstitution. All the treatments above were performed at 0–4 °C.

Chemicals used for the reconstitution were 2,3-dibromo-1,4-naphthoquinone (a gift from Dr. W. Oettmeier, Ruhr University, Bochum, Germany), 2,3-dimethyl-1,4-naphthoquinone (a gift from Dr. A. Ohsuka, Kyoto University, Kyoto), 1,4-dimethyl-9,10-anthraquinone (a gift from Dr. Y. Sakata of Osaka University, Osaka), 1,4-naphthoquinone, 2,3-dichloro-1,4-naphthoquinone, 1-chloro-9,10-anthraquinone, 2-methyl-9,10-anthraquinone, 2-ethyl-9,10-anthraquinone, 2-*tert*-butyl-9,10-anthraquinone, 1-amino-9,10-anthraquinone, 9-anthrone (Wako, Osaka), 2-methyl-1,4-naphthoquinone, 9,10-anthraquinone (Katayama, Osaka), tetramethyl-1,4-benzoquinone, 1,4-diamino-9,10-anthraquinone, benzanthrone (Tokyokasei, Tokyo), menaquinone 4, phyloquinone (Sigma, St. Louis, MO), 2,3-dimethoxy-5-methyl-1,4-benzoquinone, 1,2-naphthoquinone, 2-amino-9,10-anthraquinone, 1,2-diamino-9,10-anthraquinone, 2,6-diamino-9,10-anthraquinone, 5,12-naphthacenequinone, and 9,10-phenanthrenequinone (Aldrich, Milwaukee, WI).

**Flash Photolysis.** Flash-induced absorption changes of samples in a cuvette (10-mm light path length) were measured by use of a split-beam spectrophotometer with a time response of 1  $\mu$ s and a wavelength resolution of 3-nm half-bandwidth (Itoh & Iwaki, 1989a). The sample was excited by a flash from a frequency-doubled Nd-YAG laser (532 nm, 10 ns FWHM). The samples were kept at 6 °C by circulation of cooled water. Ascorbate (10 mM) and dichloroindophenol (0.1 mM) were added before each measurement to prereduce P700, which had been in the oxidized state after the ether treatment.

**Calculation of the Change in Free Energy of Quinones/Quinonoid Compounds on Binding at the  $Q_\phi$  Site.** The apparent dissociation constants ( $K_d$ ) between the quinones and the  $Q_\phi$  site were calculated from the dependency of the amount of the quinone-reconstituted RC on the concentration of added quinones. The amount of quinone-reconstituted RC was estimated by the extent of absorbance change at 695 nm, 50  $\mu$ s

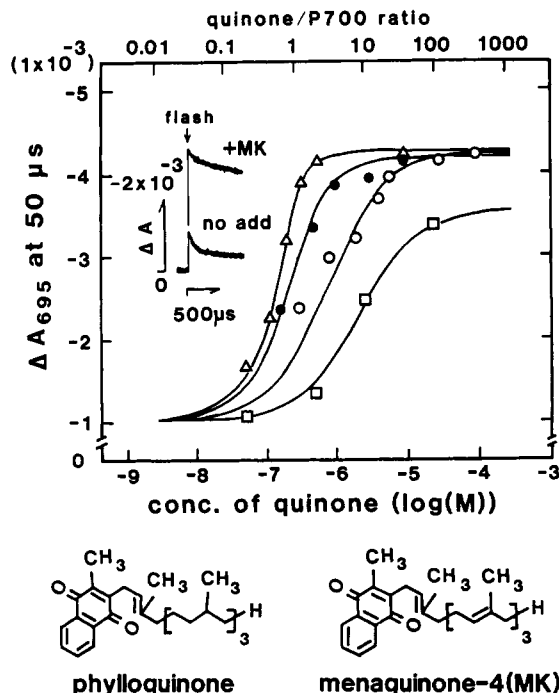


FIGURE 1: Dependencies of the extent of  $P700^+$  at 50  $\mu$ s after flash excitation on the concentration of quinones added to the incubation medium containing the ether-extracted PSI particles. Symbols shown by  $\Delta$ ,  $\bullet$ ,  $\circ$ , and  $\square$  represent data for menaquinone 4, 2,3-dimethyl-1,4-naphthoquinone, 1,4-dimethyl-9,10-anthraquinone, and 1,4-naphthoquinone, respectively. Each curve is calculated by assuming the dissociation equilibrium between quinone and RC protein. Time courses of flash-induced difference absorbance change of  $P700^+$  in the absence and presence of 0.5  $\mu$ M of menaquinone 4 (MK) are also shown in insert. The absorbance changes were measured at 695 nm, at 6  $^{\circ}$ C. The reaction mixture contained 50 mM Tris-HCl buffer, pH 7.5, 0.002 % Triton X-100, and the ether-extracted PSI particle equivalent to 0.25  $\mu$ M  $P700$ . The molecular formulas of phylloquinone and menaquinone 4 are also shown.

after the laser flash excitation. To obtain the  $K_d$  value, the dependence of the extent of  $P700^+$  on the concentration of each compound was measured, and the value was estimated by the curve-fitting program as done previously (Iwaki & Itoh, 1989). Binding free energy change  $\Delta G_t$  was calculated from the  $K_d$  value obtained experimentally according to eq 3.

## RESULTS

**Kinetics of Flash-Induced  $P700^+$  in PS I RC Containing Various Quinones and Quinonoid Compounds in Place of Phylloquinone.** In the PS I RCs whose intrinsic phylloquinone is extracted with diethyl ether, the flash excitation induced only a small extent of  $P700^+$  in a microsecond–millisecond time range (insert in Figure 1). This is due to the rapid return of an electron from  $A_0^-$  to  $P700^+$  (charge recombination) with a characteristic  $t_{1/2}$  of 35 ns (Ikegami et al., 1987; Biggins & Mathis, 1988; Itoh & Iwaki, 1988; Mathis et al., 1988). This reaction produces a triplet state,  $P700^T$ , also detected at 695 nm as the small rapid ( $t_{1/2} = 80 \mu$ s) decay. The  $t_{1/2}$  of  $P700^T$  in this preparation is slower than that usually seen in intact PS I RC probably due to the depletion of intrinsic triplet quenchers, carotenoids, by the other extraction. A small extent of  $P700^+$  decays slowly ( $t_{1/2} = 200$  ms), due to reduction either by an ascorbate–dichloroindophenol couple or by iron–sulfur centers that are reduced via an inefficient pathway from  $A_0^-$ .

The flash-induced  $P700^+$  extent in the microsecond time range was increased by addition of menaquinone 4 (insert in Figure 1). This indicates that the charge recombination reaction between  $P700^+$  and  $A_0^-$  was suppressed due to the electron transfer from  $A_0^-$  to the quinone with a half-time of

less than 1 ns [150 ps in the case of reconstituted 2-methyl-1,4-naphthoquinone according to Kim et al. (1989)]. Therefore, the initial extent of the flash-induced absorption change of  $P700^+$  is expected to be proportional to the amount of PS I RC that contains  $Q_{\phi}$  quinone. The extent observed at 695 nm at 50  $\mu$ s after the flash was plotted against the concentration of added quinones (Figure 1). This time was selected to minimize the contribution of  $P700^T$  in the estimation of  $P700^+$ . The extent increased as the increase of the concentration of added quinone (Figure 1). The dependency with menaquinone indicates that one molecule of menaquinone is necessary to fully recover the electron transfer from  $A_0^-$  to the quinone as reported in the case of phylloquinone reconstitution (Itoh & Iwaki, 1988). The dependency of increased  $P700^+$  extent on the quinone concentration was measured for each quinone. Curves calculated by assuming the dissociation equilibrium (eq 1) were fitted with these data.  $K_d$  values were estimated to be <0.01, 0.063, 0.63, and 2.0  $\mu$ M for menaquinone 4, 2,3-dimethyl-1,4-naphthoquinone, 1,4-naphthoquinone, and 1,4-dimethyl-9,10-anthraquinone, respectively.  $K_d$  values of other quinones and quinonoid compounds were also estimated by the same method.

**Effects of Hydrophobicity of *p*-Quinones Modified by Additions of Alkyl or Aromatic Groups on the Affinity to the  $Q_{\phi}$  Site.** The apparent total binding free energy change,  $\Delta G_t$  ( $= -RT \ln K_d^{-1}$ ) of *p*-quinone molecules having hydrocarbon side chains were plotted against the free energy change for partition between water and cyclohexane phases,  $\Delta G_{w-c}$  ( $= -RT \ln P$ ) in Figure 2A. The straight line in the figure was calculated by the least-squares approximation to the data with unsubstituted naphthoquinone and 2-methyl- and 2,3-dimethyl-1,4-naphthoquinone and 9,10-anthraquinone as

$$\Delta G_t = 1.1 \Delta G_{w-c} - 5.7 \text{ kcal/mol} \quad (10)$$

A distinct positive correlation between  $\Delta G_t$  and  $\Delta G_{w-c}$  is shown by the plot. This indicates that the hydrophobicity of the added compounds, represented by  $\Delta G_{w-c}$  is a major factor in determining the binding affinity represented by  $\Delta G_t$ . The data of 2,3-dimethoxy-5-methyl-1,4-benzoquinone (ubiquinone 0) falls on the line, while that of tetramethyl-1,4-benzoquinone is off the line. The slope of 1.1, obtained in Figure 2A, nearly equals 1 and seems to indicate that cyclohexane and the protein at the  $Q_{\phi}$  site show similar hydrophobicity. The  $\Delta G_t$  value of menaquinone 4 was estimated to be < -10 kcal/mol, that is, similar to that of phylloquinone (< -10 kcal/mol; Itoh & Iwaki, 1989a). Their exact  $P$  values, presumably larger than that of *tert*-butyl-9,10-anthraquinone, have not been estimated yet.

The hydrophobicity of a compound represented by its  $P$  values does not seem to be a single determinant of the affinity to the  $Q_{\phi}$  site, since the extrapolation of the line to  $\Delta G_{w-c} = 0$  still gives  $\Delta G_t$  of -5.7 kcal/mol. Therefore, some binding force,  $\Delta G_{sp}$  other than hydrophobic interaction seems to exist in the binding of these compounds at the  $Q_{\phi}$  site.

The changes of affinity on the attachment of alkyl groups or an aromatic ring to the 9,10-anthraquinone rings are shown in Figure 2B. The  $-\Delta G_t$  value of 5,12-naphthacenequinone was larger than that of 9,10-anthraquinone. On the other hand, those of alkylated anthraquinones were smaller, although the  $P$  value increased on the addition of hydrocarbon groups.

**Effects of Position and Number of Carbonyl Groups on the Affinity of Quinones and Quinonoid Compounds at the  $Q_{\phi}$  site.** *o*-Quinones, such as 1,2-naphthoquinone and 9,10-phenanthrenequinone, or quinonoid compounds with only one carbonyl group, such as 9-anthrone and benzanthrone, were also reconstituted and were shown to efficiently mediate

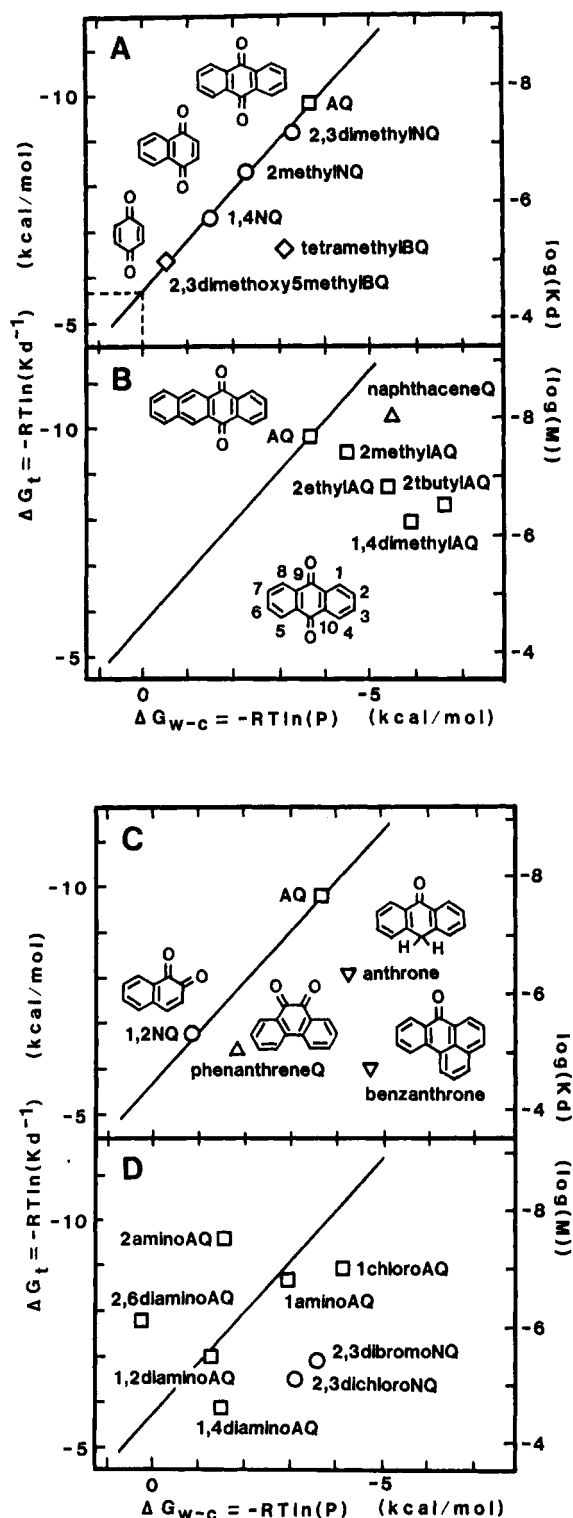


FIGURE 2: Correlation between the apparent free energy change in binding at the  $Q_\phi$  site ( $\Delta G_t (=RT \ln K_d^{-1})$ ) and the free energy change for the partition of quinone between cyclohexane and water ( $\Delta G_{w-c} (=RT \ln P)$ ). A scale for the logarithm of the apparent dissociation constant ( $\log K_d$ ) is also shown. Each  $K_d$  value was obtained in experiments similar to those in Figure 1. (A) Effect of the hydrophobicity of quinone: the straight line was obtained by a least-squares method with use of the points for unsubstituted naphthoquinone and 2-methyl- and 2,3-dimethyl-1,4-naphthoquinones and 9,10-anthraquinone. (B) Effect of the attachment of alkyl groups or an aromatic ring to anthraquinone. (C) Effect of altering the position and number of carbonyl. (D) Effect of the attachment of amino groups or halogens to quinone. The lines in (B), (C), and (D) are the same as that in (A). Symbols shown by  $\diamond$ ,  $\circ$ ,  $\square$ ,  $\Delta$ , and  $\nabla$  represent data for 1,4-benzoquinone (BQ), 1,4- or 1,2-naphthoquinone (NQ), 9,10-anthraquinone (AQ), other quinone (Q) and quinonoid compounds, respectively.

electron transfer between  $A_0$  and iron-sulfur centers as reported (Itoh & Iwaki, 1991). This indicates that both of the carbonyl groups of *p*-quinones are not necessarily required to bind at the  $Q_\phi$  site. 9-Anthrone and benzanthrone showed lower affinities than expected from their  $P$  values (Figure 2C). Steric hindrance does not explain the weaker binding of anthrone, since the molecular size of this compound is similar to that of 9,10-anthraquinone. The difference between the  $\Delta G_{w-c}$  values of anthrone and anthraquinone is calculated to be  $-0.6$  kcal/mol, while the difference between their  $\Delta G_t$  values is  $+1.8$  kcal/mol. This suggests that the deletion of a carbonyl group decreases the interaction between these compounds and the  $Q_\phi$  site. Benzanthrone showed a lower affinity. This may be due to the cooperative effects of the carbonyl deletion and the steric hindrance resulting from the addition of aromatic ring at the position opposite to the carbonyl.

The point for 1,2-naphthoquinone is nearly on the extrapolated line, while 9,10-phenanthrenequinone showed a lower affinity than that expected from the line (Figure 2C).

**Effect of Substitution by Polar Groups.** The apparent affinities of 2,3-dichloro- and 2,3-dibromo-1,4-naphthoquinones at the  $Q_\phi$  site were about 3 kcal/mol weaker than that expected from their  $P$  values (Figure 2D). A similar, lower affinity was observed in the case of 1-chloro-9,10-anthraquinone. Attachment of the electronegative groups such as chloric or bromic atoms to mother quinone compounds decreased the affinity at the  $Q_\phi$  site despite the increase in hydrophobicity.

The affinities of 2-amino- and 2,6-diamino-9,10-anthraquinone were about 2 kcal/mol higher than those expected from their  $P$  values. The affinities of 1-amino- and 1,2-diamino-9,10-anthraquinone were similar to those expected from their  $P$  values. 1,4-Diamino-9,10-anthraquinone showed about 1.5 kcal/mol lower affinity than that expected from its  $P$  value. While substitution by amino groups at the  $\beta$  position stabilized the binding, that at the  $\alpha$  position seems to lower the affinity.

## DISCUSSION

**Nonpolar Interaction between Quinones and the  $Q_\phi$  Site.** With unsubstituted naphthoquinone and methyl- and dimethylnaphthoquinones and unsubstituted anthraquinone, linear correlation was observed between the apparent binding free energy change ( $\Delta G_t$ ) and the free energy change ( $\Delta G_{w-c}$ ) required to move quinone from water into cyclohexane. This strongly suggests that the  $Q_\phi$  site is as hydrophobic as cyclohexane. Hydrophobicity of the quinones is also reported to be important for quinone binding at the bacterial  $Q_A$  site (Warncke et al., 1987; Warncke & Dutton, 1990). The binding niche is not limited to the size of naphthoquinone only. Naphthacenequinone and benzanthrone, which are much larger than anthraquinone, are still accepted; especially the former binds strongly. However, steric hindrance in binding may increase when the molecular size of a compound becomes larger than anthraquinone by addition of bulky hydrocarbon groups.

**Role of Carbonyls.** One-carbonyl quinonoid compounds bind more weakly by 2–3 kcal/mol than alkylated quinones of similar  $P$  values and molecular sizes. On the other hand, a  $\Delta G_{sp}$  value of  $-5.7$  kcal/mol is about twice that seen by the deletion of a one-carbonyl group (Figure 2A). Substitution at the 1 and 4 positions of anthraquinone by methyl groups decreased the affinity. This may indicate that the attachment of groups at the  $\alpha$  position sterically hinders the interaction of carbonyl with the protein. Substitution by amino groups at 1 and 4 positions was also not preferred. This may be explained either by steric hindrance or by intramolecular hydrogen bonding between the quinone carbonyls and the

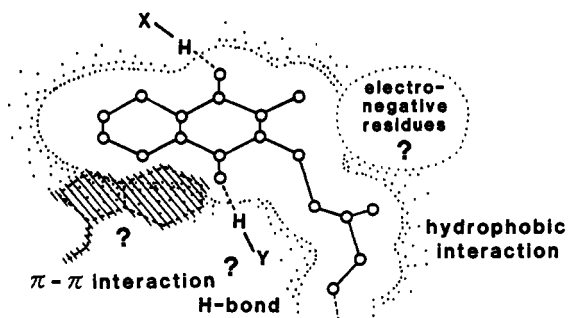


FIGURE 3: Feature of phylloquinone binding at the  $Q_A$  site in the PS I RC complex. See text for details.

$\alpha$ -amino hydrogens, which will also decrease the contribution of a carbonyl. These results indicate that the carbonyl group contributes to binding at the  $Q_A$  site.

Studies by ENDOR (Feher et al., 1985) and X-ray crystallography (Allen et al., 1988) in the *Rb. sphaeroides* RC showed that the distance between the hydrogens and the two carbonyl oxygens of  $Q_A$  quinone are 1.55 and 1.78 Å, respectively. On the other hand, quinone-reconstitution studies in the  $Q_A$  site of *Rb. sphaeroides* RC (Gunner et al., 1985) indicated that the quinone affinities are weakened by 0.6 kcal/mol and by more than 4 kcal/mol when one and both carbonyls are removed, respectively. These results suggest that the shorter H bond mainly contributes to the observed free energy change of 4 kcal/mol. The  $\Delta G_{sp}$  value of -5.7 kcal/mol observed in the PS I RC may reflect that both of the quinone carbonyls form H bonds with amino acid residues of side chains or backbone nitrogens of RC protein or may indicate some other interactions.

**Polar Interactions between Quinones and the  $Q_A$  Site.** Halogenated quinones showed lower affinity than alkylated quinones, even though their  $P$  values are similar. On the other hand, compounds substituted by amino groups at the  $\beta$  position of anthraquinone showed a stronger binding at the  $Q_A$  site than that expected from their  $P$  values. This suggests that there are some amino acid residues interacting with the polar group of the quinone, although intrinsic phylloquinone, which has no polar side chain, cannot gain the binding energy by interacting with them. Some amino acid residues in the quinone-binding niche may be negatively charged or may function as a proton donor or acceptor for an amino group of the quinone to form a H bond.

**Predicted Structure of the  $Q_A$  Site.** The molecular structure of the  $Q_A$  site may be speculated as follows (see Figure 3). The binding niche has a space that can freely accept a compound as large as an anthraquinone ring. The interior of the niche seems to be as hydrophobic as cyclohexane. The long hydrocarbon tail of phylloquinone, which will protrude from the niche, also contributes to the tight binding of these quinones to the site, as reported in the case of the  $Q_A$  site of *Rb. sphaeroides* (Warncke et al., 1987). It is not yet clear whether the difference in the structure of the tail chain induces significant difference in affinity or not. H bonding between quinone carbonyls and the protein is also expected. Existence of a negatively charged residue nearby quinone is also assumed, since the negative charge will decrease the affinities of halogenated quinones and will increase those of amino-substituted quinones.

The structure deduced above should also interpret the functional aspect. The  $E_m$  value of phylloquinone at the  $Q_A$  site is estimated to be about 0.6 V more negative than that of menaquinone in the *Rps. viridis*  $Q_A$  site (Iwaki & Itoh, 1991).  $Q^{\cdot-}$  at the  $Q_A$  site, thus, is more destabilized than in

the  $Q_A$  site. The estimated H bond of carbonyl oxygen is expected to rather stabilize  $Q^{\cdot-}$ , as seen in the  $Q_A$  site. Therefore, it hardly interprets the extremely negative  $E_m$  of quinone at the  $Q_A$  site. The negative charge nearby  $Q_A$  quinone may partially interpret the negative  $E_m$  but seems to be insufficient.

An additional possibility to combine the structure and function may be the  $\pi$ - $\pi$  electronic interaction between aromatic quinone rings and the aromatic residues like tryptophan, phenylalanine, or tyrosine, which are abundant in the PS I RC proteins (Kirsch et al., 1986). The interaction will increase the electron density on the carbonyl oxygens of  $Q_A$  quinone. Then,  $Q_A^{\cdot-}$  will be destabilized. The methyl group at the ortho position to the quinone carbonyl may also contribute to expand the  $\pi$  system through the pseudo- $\pi$ -conjugation between a carbonyl oxygen and methyl hydrogen. These effects may explain the observed order of apparent affinities, i.e., naphthacenequinone > anthraquinone > methylanthraquinone > naphthoquinone, since the interaction will increase as the expansion of the  $\pi$  system. The  $\pi$ - $\pi$  interaction, thus, may interpret both the functional and structural aspects of the  $Q_A$  site if the special arrangement of the quinone rings and aromatic rings of amino acid residues, as suggested in Figure 3, may be achieved. Further work is required to obtain the more reliable structure.

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**Registry No.** 2,3-Dibromo-1,4-naphthoquinone, 13243-65-7; 2,3-dimethyl-1,4-naphthoquinone, 2197-57-1; 1,4-dimethyl-9,10-anthraquinone, 1519-36-4; 1,4-naphthoquinone, 130-15-4; 2,3-dichloro-1,4-naphthoquinone, 117-80-6; 1-chloro-9,10-anthraquinone, 82-44-0; 2-methyl-9,10-anthraquinone, 84-54-8; 2-ethyl-9,10-anthraquinone, 84-51-5; 2-*tert*-butyl-9,10-anthraquinone, 84-47-9; 1-amino-9,10-anthraquinone, 82-45-1; 9-anthrone, 90-44-8; 2-methyl-1,4-naphthoquinone, 58-27-5; 9,10-anthraquinone, 84-65-1; tetramethyl-1,4-benzoquinone, 527-17-3; 1,4-diamino-9,10-anthraquinone, 128-95-0; benzanthrone, 82-05-3; menaquinone 4, 863-61-6; phylloquinone, 84-80-0; 2,3-dimethoxy-5-methyl-1,4-benzoquinone, 605-94-7; 1,2-naphthoquinone, 524-42-5; 2-amino-9,10-anthraquinone, 117-79-3; 1,2-diamino-9,10-anthraquinone, 1758-68-5; 2,6-diamino-9,10-anthraquinone, 131-14-6; 5,12-naphthacenequinone, 1090-13-7; 9,10-phenanthrenequinone, 84-11-7.

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## Structure of the Membrane-Bound Protein Photosynthetic Reaction Center from *Rhodobacter sphaeroides*<sup>†</sup>

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**ABSTRACT:** The structure of the photosynthetic reaction center (RC) from *Rhodobacter sphaeroides* was determined at 3.1-Å resolution by the molecular replacement method, using the *Rhodospseudomonas viridis* RC as the search structure. Atomic coordinates were refined with the difference Fourier method and restrained least-squares refinement techniques to a current *R* factor of 22%. The tertiary structure of the RC complex is stabilized by hydrophobic interactions between the L and M chains, by interactions of the pigments with each other and with the L and M chains, by residues from the L and M chains that coordinate to the Fe<sup>2+</sup>, by salt bridges that are formed between the L and M chains and the H chain, and possibly by electrostatic forces between the ends of helices. The conserved residues at the N-termini of the L and M chains were identified as recognition sites for the H chain.

The photosynthetic reaction center (RC) is a transmembrane protein complex that carries out the light-induced charge separation that is the first step in photosynthesis. The structure of the RC from *Rhodospseudomonas viridis* was determined at 2.3-Å resolution (Deisenhofer & Michel, 1989). Chang et

al. (1986) and Allen et al. (1986) used the coordinates of the *Rps. viridis* reaction center to solve the structure of the RC from *Rhodobacter sphaeroides* R-26 by the technique of molecular replacement. The RC from *Rb. sphaeroides* R-26 consists of three protein subunits, the L, M, and H chains. The L and M chains are transmembrane proteins with homologous structures and amino acid sequences. Each chain has five transmembrane helices, designated A, B, C, D, and E (Deisenhofer et al., 1985). The L and M chains are related to each other in the complex by a local 2-fold axis. Embedded in the L and M chains are nonprotein cofactors: four bacteriochlorophylls, two of which form the so-called "special pair" (BC<sub>LP</sub> and BC<sub>MP</sub>) and two that are monomeric "accessory" bacteriochlorophylls (BC<sub>LA</sub> and BC<sub>MA</sub>), two bacteriopheophytins (BP<sub>L</sub> and BP<sub>M</sub>), two quinones (Q<sub>A</sub> and Q<sub>B</sub>), and a

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