Electron transfer in spinach photosystem I reaction center containing benzo-, naphtho- and anthraquinones in place of phylloquinone

Masayo Iwaki and Shigeru Itoh

National Institute for Basic Biology, 38 Nishigonaka, Myodaijicho, Okazaki 444, Japan

Received 26 July 1989

In spinach photosystem I particles, constituent phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone; vitamin K_1) was replaced by 14 different benzo-, naphtho- and anthraquinones. All of the quinones tested suppressed the nanosecond charge recombination between the reduced electron acceptor chlorophyll *a*, A_0^-, and the oxidized primary donor, $P700^+$, suggesting that they replace the function of the electron acceptor A_1 and rapidly oxidize A_0^-. The binding affinity of these quinones for the photosystem I reaction center increased in the order of benzoquinone < naphthoquinone < anthraquinone. The phytyl tail of phylloquinone was also shown to increase the binding affinity. The flash-induced kinetics of $P700^+$ varied independently of the dissociation constants. Only the quinones (including phylloquinone) which are estimated to exhibit an in situ redox midpoint potential (E_m) value between those of A_0 and the iron-sulfur center F_X fully replaced the function of A_1. These results confirm that A_1 is phylloquinone and indicate that the phylloquinone-binding site in the photosystem I reaction center gives an environment in which the E_m value for the semiquinone–quinone couple is significantly lower than that in the Q_A site in the reaction center of purple bacteria.

Quinone; Phylloquinone; Vitamin K_1; Photosystem I; Reaction center; Electron transfer; Photosynthesis

1. INTRODUCTION

In the photosystem I reaction center of oxygenic photosynthetic organisms, absorption of light oxidizes a special chlorophyll *a* species, P700, and initiates a series of electron transfer steps as follows [1]:

$$P700 \underset{<3\text{ ps [2–5]}}{\rightleftharpoons} A_0 \xrightarrow[35\text{ ps [3–5]}]{} A_1 \xrightarrow[15\text{ or }200\text{ ns [6,7]}]{} F_X \longrightarrow$$

$$F_B, F_A \; (\rightarrow\rightarrow\rightarrow \text{NADP})$$

35 ns [3,5,8–11]

Correspondence address: S. Itoh, National Institute for Basic Biology, 38 Nishigonaka, Myodaijicho, Okazaki 444, Japan

Abbreviations: P700, photosystem I primary electron donor chlorophyll *a*; A_0 and A_1, photosystem I primary electron acceptors; Q_ϕ site, photosystem I phylloquinone (and herbicide)-binding site; Q_A and Q_B (sites), primary and secondary electron acceptor quinones (binding sites) in PS II or purple bacterial reaction center; DMF, dimethylformamide; K_d, dissociation constant; E_m, midpoint potential; $E_{1/2}$, a half wave reduction potential polarographically measured; $t_{1/2}$, a half decay time

where A_0 is the electron acceptor chlorophyll *a*-690 [3–5,11], and A_1 the primary electron acceptor, F_X, F_A or F_B iron-sulfur center X, A or B. The chemical identity of A_1 is presumably one of two phylloquinone (2-methyl-3-phytyl-1,4-naphthoquinone; vitamin K_1) molecules contained in the PS I reaction center [1,12,13]. However, this idea is not in agreement with the evidence that the destruction of phylloquinone by UV light did not affect the P700 turnover [14,15].

Extraction of phylloquinone from PS I reaction centers by ether [10,16,17] or by hexane-methanol [8] stops oxidation of A_0^- by A_1 and enhances the charge recombination reaction between A_0^- and $P700^+$. $P700^+$ decays rapidly after the flash excitation, and only a small extent of $P700^+$ can be detected in the microsecond to millisecond time range in these preparations. This reaction leads to the light generation of $P700^T$ [9,10,16,17] or P700* which leads to delayed fluorescence [10]. Addition of phylloquinone or menadione reconstitutes electron transfer from A_0^- to iron-sulfur centers [17],

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00145793/89/$3.50

and extends the life time of $P700^+$ into the microsecond to millisecond time range by suppressing the charge recombination [8–10,16,17]. These results strongly suggest that A_1 is phylloquinone.

If phylloquinone serves as A_1, which functions to produce strong reducing power, its binding site in the PS I reaction center (designated as the Q_ϕ site in the previous study from its herbicide-binding feature [18]) should provide an environment which enables it to function at a redox potential significantly lower than those of the primary (Q_A) and the secondary (Q_B) electron acceptor quinones in reaction centers of PS II and purple bacteria. It is also interesting to know how the Q_ϕ site distinguishes phylloquinone from plastoquinone which functions in PS II.

In this work the function of quinone as A_1 is studied in the spinach PS I reaction center preparation which contains artificial benzo-, naphtho- or anthraquinone instead of the native phylloquinone. Here we report that the efficiency of quinone to function as A_1 depends both on its structure and redox properties.

2. MATERIALS AND METHODS

Lyophilized photosystem I particles, obtained by treating spinach chloroplasts with digitonin, were extracted twice with diethyl ether saturated with water to a 50% level, followed by one extraction with dry diethyl ether: this procedure completely extracted the 2.2 phylloquinone molecules/P700 contained in the original PS I particles [16]. The phylloquinone-depleted particles were also depleted of about 85% of the antenna chlorophyll complement and all carotenoids [16–19]. However, P700, A_0, F_X, F_B and F_A were almost unaffected [11,16–19]. The extracted particles were dispersed in 50 mM glycine-OH buffer, pH 10, and then diluted in 50 mM Tris-Cl buffer, pH 7.5, containing 0.3% (v/v) Triton X-100. After 30 min incubation, grayish undissolved materials (mainly PS I light harvesting chlorophyll proteins and cytochrome b/c_1 complex) were eliminated by centrifugation. The clear supernatant was about 50 times diluted with 50 mM Tris-Cl buffer, pH 7.5, containing 30% (v/v) glycerol to give a final P700 concentration of 0.25 μM, and used for reconstitution and measurements.

To reconstitute quinones, the suspension of the extracted PS I particles was incubated for a day at 0°C in the dark with various quinones dissolved in ethyl alcohol, hexyl alcohol or dimethyl sulfoxide. The quinone-reconstituted reaction centers were stable for several days when stored below 0°C. More than 70% of the PS I reaction centers were reconstituted by this method [17]. 10 mM ascorbate and 0.1 mM dichloroindophenol couple were added to the reaction medium to provide a second time scale reduction of the small amount of $P700^+$ not rapidly reduced by A_0 or A_1. Quinones used as candidates to reconstitute A_1 were 2,6-diamino-9,10-anthraquinone, ubiquinone-0 (Aldrich, Milwaukee, WI, USA), 9,10-anthraquinone, menadione (Katayama, Osaka, Japan), 2-phenyl-1,4-benzoquinone, phylloquinone (Sigma, St. Louis, MO, USA), duroquinone, 1,4-naphthoquinone, 1-nitro-9,10-anthraquinone (Tokyokasei, Tokyo, Japan), 1-amino-9,10-anthraquinone, 2,3-dichloro-1,4-naphthoquinone, 2-methyl-9,10-anthraquinone (Wako, Osaka, Japan), ubiquinone-10 and plastoquinone-9 (kind gifts from Drs S. Okayama and Y. Isogai of Kyusyu University, Fukuoka, Japan).

Activity of the reconstituted PS I particles was assayed by measuring the flash-induced absorption change of P700 at 695 nm in a split-beam spectrophotometer [16–18] at 6°C. The intensity of actinic flash (532 nm, 10 ns FWHM, 0.7 Hz) from a frequency doubled Nd-YAG laser (Quanta-Ray, DCR-2-10), was attenuated to excite about a quarter of reaction centers to avoid sample damage. Signals were averaged between 32 and 128 scans in each case as was required.

3. RESULTS AND DISCUSSION

3.1. *Kinetics of flash-induced $P700^+$ in PS I reaction center containing various quinones*

In PS I reaction centers in which native phylloquinone is extracted with diethyl ether, only a small extent of $P700^+$ was detected in the microsecond to millisecond time range (traces of no additions in fig.1a–c). This is due to the rapid return of an electron on A_0^- to $P700^+$ with a characteristic $t_{1/2}$ of 35 ns [8–11]. This reaction produces a triplet state, $P700^T$ ($t_{1/2}$ = 80 μs) also detected at 695 nm (fig.1a–c). A small extent of $P700^+$ decays slowly ($t_{1/2}$ = 10–100 ms), due to the reduction either by an ascorbate-dichloroindophenol couple or by photoreduced iron-sulfur centers.

The flash-induced $P700^+$ extent in the microsecond to millisecond range was increased when various benzo-, naphtho- and anthraquinones, including those known as inhibitors in PS II [21], were substituted for intrinsic phylloquinone in the PS I reaction center (fig.1a–c). This indicates that all of these compounds can oxidize A_0^-. The concentration of each quinone used in fig.1 was adjusted to provide the maximum level of reconstitution.

3.2. *Binding affinity of quinones*

The extent of $P700^+$ at 50 μs after flash excitation depended on the concentration of added quinone (fig.2). Theoretical curves, calculated with dissociation constant (K_d) values of 7.5, 2.5 and 0.015 μM, fitted well to the data for duroquinone, 1,4-naphthoquinone and 9,10-anthraquinone,

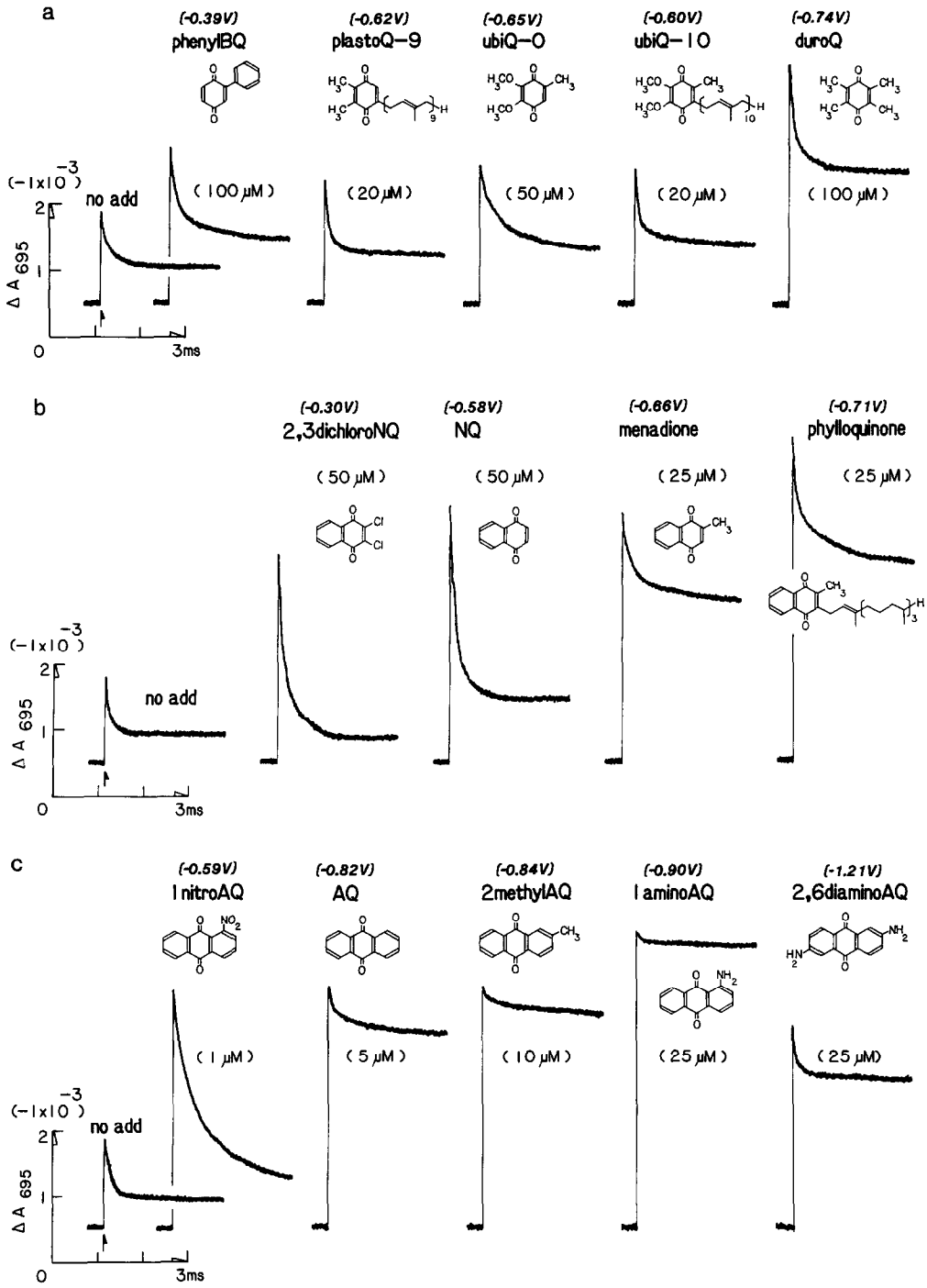

Fig.1. Flash-induced absorption change of P700 in the presence of various benzo- (a), naphtho- (b), and anthraquinones (c) in phylloquinone-depleted PS I particles measured at 695 nm. The concentration of added quinone is shown in parentheses. Redox properties of quinones are represented by their $E_{1/2}$ values measured in DMF [23,26,27] as shown in brackets. The $E_{1/2}$ values, referred to the saturated calomel electrode, are comparable to those referred to the standard hydrogen electrode by adding 0.24 V. BQ, NQ, AQ, duroQ, plastoQ and ubiQ represent 1,4-benzoquinone, 1,4-naphthoquinone, 9,10-anthraquinone, duroquinone, plastoquinone and ubiquinone, respectively. Filled arrows indicate the onset of the actinic laser flash.

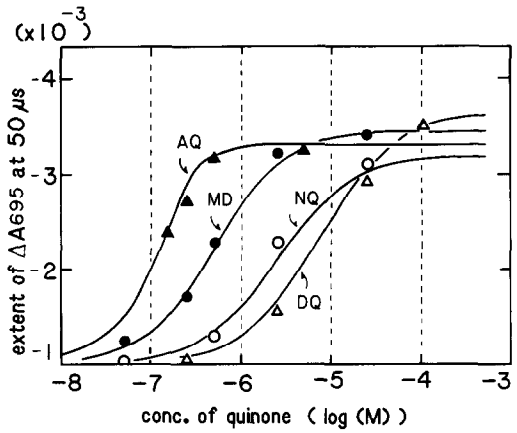

Fig.2. Dependence of the extent of photo-oxidized $P700^+$ at 50 μs after flash excitation, on the concentration of added quinone. Each curve is calculated by assuming the one point dissociation equilibrium between reaction center protein and quinone. Estimated dissociation constants are 7.5, 2.5, 0.35 and 0.015 μM for duroquinone (DQ), 1,4-naphthoquinone (NQ), menadione (MD) and 9,10-anthraquinone (AQ), respectively. Other conditions are as described in fig.1.

respectively. This indicates that increasing the number of aromatic rings of the quinone leads to lower K_d values and hence tighter binding. K_d values were estimated to be 0.35 and 0.0025 μM for menadione and phylloquinone, respectively [18]. The attachment of a hydrocarbon tail seems to result in tighter binding when the K_d values of 1,4-naphthoquinone, menadione and phylloquinone were compared. The more hydrophobic quinones bind to the reaction center more tightly as reported in the bacterial Q_A site [22]. However, plastoquinone-9 and ubiquinone-10, which are known to function in PS II and bacterial reaction centers [1], respectively, were less effective, although they have long hydrocarbon tails. These quinones may not properly bind to the Q_ϕ site. This remains to be studied.

3.3. *Kinetics of $P700^+$ and redox properties of quinone*

Kinetic patterns of the flash-induced $P700^+$ seemed to vary depending on the redox potential of the reconstituted quinone given by the polarographic $E_{1/2}$ value of the semiquinone$^{\cdot -}$/quinone couple in DMF. This suggests that the midpoint potential value of quinone at the Q_ϕ site (the in situ E_m value), with respect to those of A_0 and F_X, is crucial in determining the reaction kinetics of the quinone, since the E_m in situ is roughly related to $E_{1/2}$ [23].

First of all, there is a group (called type B in fig.3) of quinones which induced the high extent of flash-oxidized $P700^+$ followed by a slow decay ($t_{1/2}$ = 10–100 ms), as already reported in the case of phylloquinone [17]. The slow decay time corresponds to the re-reduction of $P700^+$ by iron-sulfur centers F_A or F_B [17]. Phylloquinone and most anthraquinones (except 1-nitro-9,10-anthraquinone and 2,6-diamino-9,10-anthraquinone) belong to this group. These quinones appear to fully mediate electron flow from A_0^- to F_X and then to F_B and F_A (Iwaki, M. and Itoh, S., in preparation); i.e. they reconstituted the function of A_1. The E_m values of these quinones are expected to be intermediate between those of the A_0 and F_X.

Quinones of the A type are typified by the case of 2,6-diamino-9,10-anthraquinone which has a lower $E_{1/2}$ value. In this case, the initial extent of flash-induced $P700^+$ and the extent of the slow decay phase were small (fig.1c) even at the concentration (25 μM) much higher than the 0.8 μM K_d value. Quinones of type A may be incompletely reduced by A_0^- most probably because the E_m of the quinone is comparable to or lower than that of A_0.

Quinones of the type C are typified by duroquinone, 1,4-naphthoquinone, menadione and 1-nitro-9,10-anthraquinone. These, like those of the B type, promote a high extent of the flash-induced $P700^+$. However, quinones of the C type differ in the decay of $P700^+$ which is composed of fast ($t_{1/2}$ = 0.3–1 ms) and slow ($t_{1/2}$ = 30–100 ms) phases. The extent of the latter decreased with the raising of the $E_{1/2}$ value of the tested quinone. This may be explained if these quinones have E_m values comparable with that of F_X and can only partially reduce F_X and therefore, F_B and F_A. In this case, it is expected that the remaining semiquinone$^{\cdot -}$ then will directly reduce $P700^+$ as the fast phase.

The last type (type D) is characterized by the high extent of flash-induced $P700^+$ which decays rapidly ($t_{1/2}$ = 300 μs) with almost no increase of the slow decay phase, as seen in the case of 2,3-dichloro-1,4-naphthoquinone (fig.1b). This high potential quinone does not seem to reduce F_X. In this case, the semiquinone$^{\cdot -}$/quinone couple is estimated to have an E_m value too high to reduce

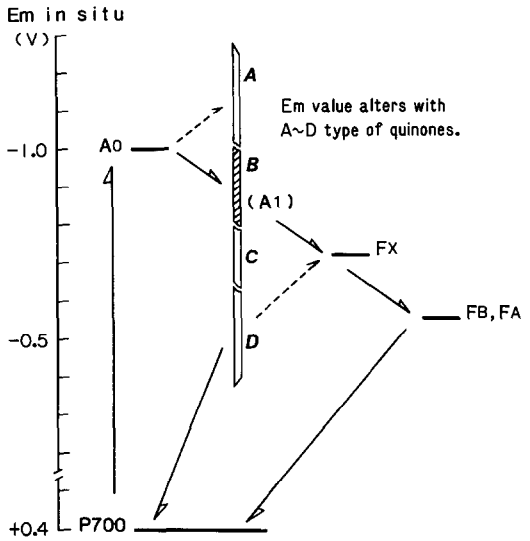

Fig.3. Energy diagram and estimated electron transfer pathways in PS I reaction centers containing various quinones with different E_m values of semiquinone$^{\cdot -}$/quinone couple (A, B, C and D types) instead of intrinsic phylloquinone (see text for details).

F_X and so the semiquinone$^{\cdot -}$ formed will reduce $P700^+$ directly with the fast rate as observed.

4. CONCLUSION

4.1. *Quinone structure and binding affinity for the Q_ϕ site*

Various quinones bind to the PS I Q_ϕ site instead of the native phylloquinone and function as A_1. This gives strong support to the conclusion that A_1 is phylloquinone and indicates that the quinone specificity of the site is not strict. The phytyl tail or naphthoquinone ring of phylloquinone, does not seem to be essential to function as A_1, although these properties contribute to the tight binding of phylloquinone to the Q_ϕ site with a significant contribution from hydrophobic interactions as reported for the bacterial Q_A site [22].

4.2. *Redox properties of quinones and their ability to function as A_1*

We propose that the thermodynamic relationship between the quinone and its reaction partners can be grouped into four (A, B, C and D) types depending on the E_m values of the semiquinone$^{\cdot -}$/quinone couple in the Q_ϕ site (fig.3) with respect to those of A_0 and F_X. The E_m value of A_0 may be similar to that of chlorophyll *a* reduction which is at about -1.0 V [24]. The E_m of F_X is reported to be -0.705 mV [25]. It is apparent that the E_m values of the quinones in the Q_ϕ site are parallel to the $E_{1/2}$ values in DMF. The quinones of the type B, which are characterized by the wide range of $E_{1/2}$ from -0.74 to -0.90 V, fully function as A_1. Benzoquinones, on the other hand, are rather unfavorable to function as A_1 because of their high E_m values.

The in situ E_m value of phylloquinone (A_1) can be estimated to be around -0.9 V (i.e. intermediate between A_0 and F_X). The E_m values are 0.4 V lower than the $E_{1/2}$ values in DMF. Moreover, the value for phylloquinone is 0.8 V lower than that of the similarly structured menaquinone of the bacterial Q_A site [23,26,27]. Menaquinone differs from phylloquinone only in the structure of the hydrocarbon tail. It is of some interest that we have shown that menaquinone functions as A_1 in the PS I Q_ϕ site (Iwaki, M. and Itoh, S., unpublished data). These observations indicate that the stability of semiquinone$^{\cdot -}$ is significantly lower at the Q_ϕ site than at the Q_A site.

Finally our results indicate that the rate of electron transfer at the Q_ϕ site seems to be mainly dependent on the E_m in situ of the semiquinone$^{\cdot -}$/quinone couple but not significantly on the structure of quinone. This confirms the conclusion of the quinone-reconstitution studies at the Q_A site in the *Rb. sphaeroides* reaction center by Dutton and co-workers [22,23,28,29]. The quinone-reconstitution and replacement studies have provided information about the dynamic relationship between the structure and function of the purple bacterial reaction center [22,23,28–31]. This kind of approach can now be adapted to green plant PS I reaction center complex whose tertiary structure still remains to be characterized.

Acknowledgements: The authors thank Dr Y. Fujita of NIBB and Professor P.L. Dutton of Univ. Pennsylvania for their stimulating discussions, and Dr J. Eaton-Rye for reviewing the manuscripts.

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