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Vitamin K_1 (phylloquinone) restores the turnover of FeS centers in the ether-extracted spinach PS I particles

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The effects of vitamin K_1 (phylloquinone) addition on the turnover of the FeS centers in photosystem I photochemistry were studied in diethyl ether-extracted spinach photosystem I particles. Reconstitution of one molecule of vitamin K_1/reaction center was sufficient to suppress the charge recombination between the oxidized reaction center chlorophyll $P700^+$ and the reduced electron acceptor intermediate chlorophyll *a* and to restore the turnover of FeS centers in the ether-extracted particles. This strongly suggests that reconstituted vitamin K_1 functions as the primary electron acceptor A_1 and exhibits a redox midpoint potential low enough to reduce the FeS centers. The quinone binding site in photosystem I, which enables vitamin K_1 to show such a low redox potential, seems to be more hydrophobic than those in reaction centers of photosystem II or purple bacteria.

Iron sulfur center; Quinone; Phylloquinone; Vitamin K_1; Photosystem I; Charge recombination

1. INTRODUCTION

Phylloquinone (VK_1), which was shown by Takahashi et al. [1] to be contained in photosystem I reaction center core polypeptides, has been suggested to be the primary stable electron acceptor A_1 [2–6] which mediates electron transfer between the electron acceptor intermediate chlorophyll *a* (A_0) and the iron sulfur centers, F_X, F_A and F_B.

We have shown that the VK_1 in PS I particles can be almost completely extracted by treatment with diethyl ether without damaging P700, A_0 or the iron sulfur centers [7]. The extraction increases the rate of charge recombination between $P700^+$ and A_0^-, a reaction which takes place with a decay half time of about 30 ns [8], and which results in the depressed extent of $P700^+$ detected in the microsecond or longer time range after the flash excitation [7,8]. The nanosecond charge-recombination reaction was suppressed by the reconstitution of VK_1 or menadione (VK_3) to the ether-extracted PS I particles [7,8]. These results were recently confirmed in the hexane-methanol extracted *Cynechocystis* PS I particles [9]. Enhancement of the charge-recombination rate by the VK_1 extraction with ether was also confirmed by the enhanced nanosecond delayed fluorescence from P700 which is produced in the charge-recombination reaction and is suppressed by VK_3-reconstitution [10]. These results strongly suggest that VK_1 works as the primary electron acceptor A_1 in the PS I reaction center and is rapidly oxidizing A_0^- according to the reaction scheme below which was formulated based on these results and those of picosecond spectroscopy [11–13].

$$\underset{\text{delayed fl. [10]}}{P700\ A_0A_1} \overset{h\nu}{\rightleftharpoons} P700^*\ A_0A_1 \underset{30\text{ ns [8–10]}}{\rightleftharpoons} P700^+\ A_0^-A_1 \overset{13\text{ ps [11]}}{\longrightarrow} P700^+\ A_0A_1^- \overset{35\text{ ps [11–13]}}{}$$

In subsequent reactions, A_1^- is assumed to reduce iron sulfur center F_X which probably mediates electrons to F_B and F_A centers as follows [5,6].

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Abbreviations: PS I, photosystem I; VK_1, vitamin K_1 (phylloquinone); A_0 and A_1, photosystem I electron acceptor intermediate chlorophyll *a* and the primary electron acceptor, respectively; E_m, redox midpoint potential

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$P700^+ A_0A_1^-F_XF_BF_A \longrightarrow P700^+ A_0A_1F_X^-F_BF_A \longrightarrow$

$P700^+ A_0A_1F_XF_BF_A^-$

However, no evidence has yet been reported that supports the idea that reduced VK_1 can lead to the reduction of these low potential F_X (E_m = −705 mV [14]), F_B (−590 mV [15]) and F_A (−550 mV [16]) centers.

It is also important to note that there are some arguments against the function of VK_1 as A_1. As we have shown [7] the extraction of VK_1 did not totally stop the light-induced reduction of FeS centers at 10 K. The flash yield of FeS reduction was found to suppress only 50% by the extraction of VK_1 at 15 K [17]. Another argument comes from the fact that the attempted destruction of VK_1 by UV light did not inhibit PS I photochemistry [18,19].

In the present study we show that about 1 VK_1 molecule/P700 restores the turnover of FeS centers and suppresses the direct charge recombination between $P700^+$ and A_0^-.

2. MATERIALS AND METHODS

Lyophilized PS I particles (about 20 mg dry wt) obtained by treating spinach chloroplasts with digitonin were extracted twice with 40 ml ice-cooled dry diethyl ether [7] and then dispersed in 10 mM Tris buffer, pH 10.0. The suspension was diluted 2–3-fold with 10 mM Tris buffer (pH 7.5) followed by addition of Triton X-100 to give a final concentration of 0.1%. After a 30 min incubation, grayish undissolved materials were eliminated by a centrifugation at 10000 × *g* for 1 min. The clear supernatant was dissolved in 10 mM Tris buffer, pH 7.0, containing 20–50% glycerol. This treatment extracted about 2.2 VK_1 molecules/P700 and about 60% of antenna chlorophylls; however, there was no P700 lost from the preparation. Similarly A_0 and the FeS centers (F_X, F_A and F_B) were almost unaffected [7]. The extraction method using dry ether is essentially the same as that used to obtain PS I particles with a smaller antenna size (8–11 chlorophyll *a*/P700) [20–22] using water-containing ether, but had less damage on FeS centers [7]. In the VK_1-reconstitution experiment, the ether-extracted PS I particles were incubated for 1 day at 0°C in the dark after vigorously mixing with various amounts of VK_1 dissolved in ethanol. Chlorophylls and P700 content were determined as described [7].

The flash-induced absorption change was measured by a split-beam spectrophotometer (time response of 1 μs) at 7°C [7]. Intensity of the flash light from a frequency doubled Nd-YAG laser (532 nm, 10 ns FWHM) was attenuated to excite about a half of reaction centers. This was done to avoid damage of the sample during measurements (16–64 signals are averaged in each case).

3. RESULTS

3.1. *Suppression of the charge recombination and the recovery of slow decay phase of $P700^+$ by VK_1-reconstitution in ether-extracted PS I particles*

In the VK_1-depleted PS I particles, the $P700^+$ formed by the laser excitation is assumed to decrease rapidly by a charge recombination between $P700^+$ and A_0^- [8–10]. Therefore, the flash-induced absorption change of P700 detectable in a ms time range is only about 20% of that expected (fig.1). In the ether-extracted PS I particles preincubated with VK_1, flash-excitation induced the larger extent of $P700^+$ in this time range as reported [7]. The VK_1-reconstitution decreased the submillisecond rapid phase with a decay time of 320 μs (mainly decay of the $P700^T$ state [7,8]) and

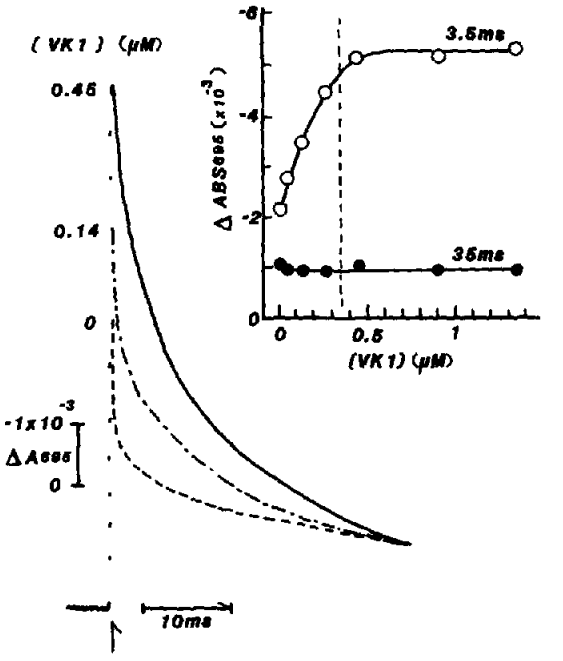

Fig.1. The effect of VK_1 reconstitution on the laser flash induced absorption change of $P700^+$ measured at its 695 nm peak in ether-extracted PS I particles. Concentrations of VK_1 added during preincubation were shown in the figure. The insert to the figure shows the effect of VK_1 concentration on the extent of $P700^+$ at 3.5 and 35 ms after the laser excitation. The dashed vertical line indicates the concentration of PS I reaction center (P700) used. The reaction mixture contained 10 mM Tris-Cl buffer, pH 7.5, 50% glycerol, 100 μM dichloroindophenol, 10 mM ascorbate, PS I particles equivalent to 0.35 μM P700 and various concentrations of VK_1.

increased the slower decay phases with apparent decay half times of 3 and 12 ms (sometimes more than 20 ms). The slowest reduction phase of $P700^+$ displaying a decay time of longer than 100 ms, mainly due to the direct reduction of $P700^+$ by dichloroindophenol and ascorbate couple, was not affected by VK_1.

Fig.1 insert shows the dependence of the extent of the slow decay phase of $P700^+$ measured at 3.5 ms after the laser excitation on the concentration of VK_1. The effects of VK_1 approached saturation above 0.5 μM. Almost 1 VK_1/P700 was sufficient for the stabilization of $P700^+$ in this time range as reported in the hexane-methanol extracted preparation [9]. In at least 70% of reaction centers, VK_1 reconstitution was estimated to restore the ms decay phase of $P700^+$ from the measurement at the higher laser intensity (not shown). These results suggest that the dissociation constant between VK_1 and its binding site is less than 10^{-8} M; this value is regarded as an upper limit because the actual VK_1 concentration in the outer medium must be much lower than calculated due to the binding of the very water-insoluble VK_1 to the PS I particles.

3.2. *Turnover of iron sulfur centers (P430) in the reconstituted system*

The recovery of the 3–12 ms decay phases of $P700^+$ suggests that the reducing equivalents transferred to VK_1 remain somewhere in the reducing side of PS I before returning to $P700^+$. When P700 was monitored at the 695 nm peak in the VK_1-reconstituted particles, addition of an electron acceptor benzyl viologen (E_m = −360 mV) slightly depressed the initial extent just after the flash excitation but elevated the extent at a longer time range (fig.2A, lower curves). The smaller fast decay phase, observed in less than 1 ms, seems to be mainly due to the decay of $P700^T$. This effect is

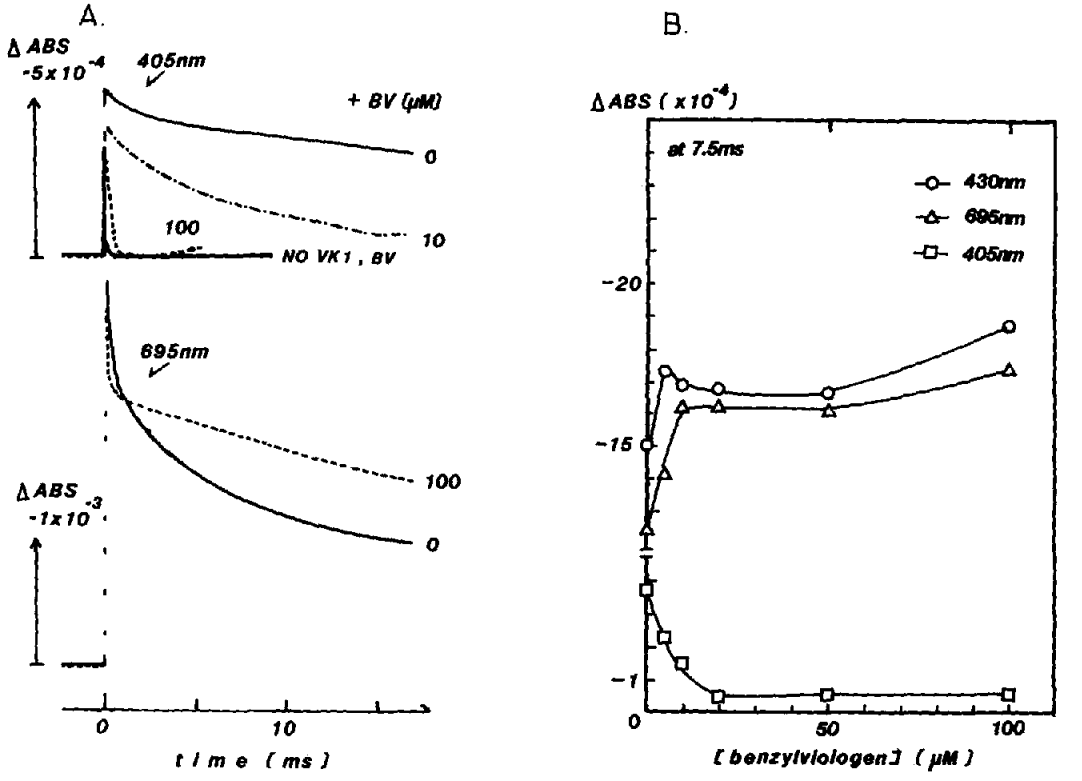

Fig.2. (A) The effect of benzyl viologen on the kinetics of flash-induced absorbance changes at 695 nm (lower) and 407 nm (upper) in the VK_1-reconstituted ether-extracted PS I particles. (B) The effect of benzyl viologen concentration on the extent of the flash-induced absorption change at 7.5 ms in the VK_1-reconstituted ether-extracted PS I particles. Concentration of VK_1, 1.35 μM. Other conditions were similar to those in fig.1.

expected if benzyl viologen competes with $P700^+$ in accepting electrons from the reducing side of PS I. At 405 nm (an isosbestic wavelength of P700) a slow decay phase, which was not observed in the ether-extracted particles, was induced after the VK_1 reconstitution (fig.2A, upper curves). Its decay rate was almost similar to the slow decay phase at 695 nm. Addition of benzyl viologen significantly accelerated the decay rate of the 405 nm phase with a small effect on the initial amplitude. This indicates that the flash activation leads to the reduction of a component which shows an absorption change at 405 nm and which has an E_m value low enough to reduce benzyl viologen in the VK_1-reconstituted particles.

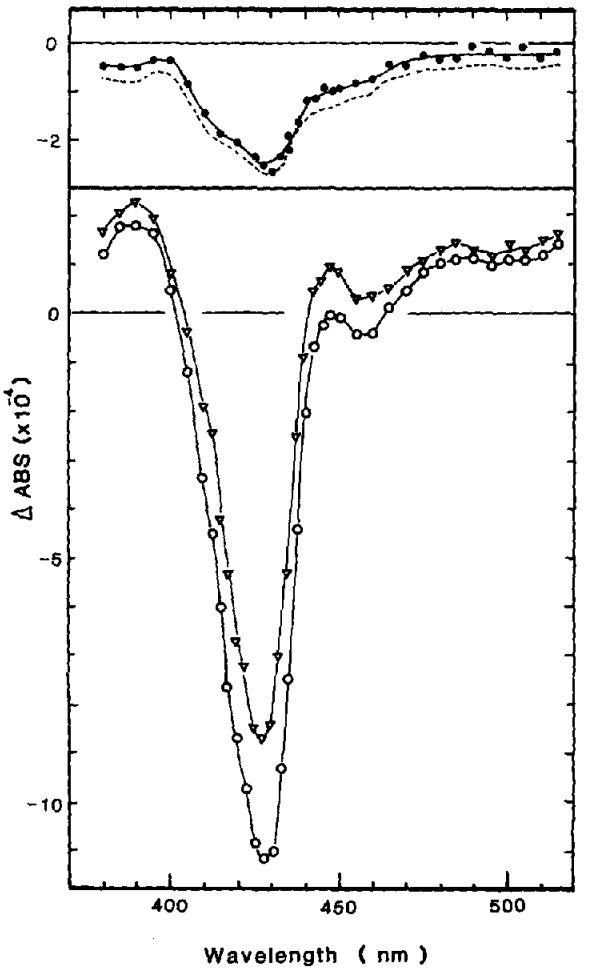

Fig.3. The flash-induced difference absorption spectra in the VK_1-reconstituted ether-extracted PS I particles in the presence of 50 μM benzyl viologen. (○, △) Absorbance changes at 0.5 and 5 ms after flash excitation; (●) fast decay component (difference between open and closed circles). The dotted line represents the difference spectrum of the 'fast component' calculated by subtracting the contribution of absorbance change due to $P700^+$ and by extrapolating to time zero. Other conditions were similar to those in fig.2.

Fig.2B shows the effect of benzyl viologen concentration on the extent of absorbance change measured in the VK_1-reconstituted particles at 695, 405 and 430 nm, 7.5 ms after the laser excitation. The increase of the $P700^+$ change at 695 nm and the decrease of the 405 nm change were observed in a similar concentration range of benzyl viologen. The extent at 430 nm responded like a mixture of these two. These effects indicate that the electron flow from the 405 and 430 nm absorbing component to $P700^+$ is suppressed by benzyl viologen which leads to the stabilization of $P700^+$. Fig.3 shows the difference absorption spectra obtained at 0.5 ms, chosen to eliminate contribution of $P700^T$ state which decays with 320 μs half time, and at 5 ms after the flash excitation in the VK_1-reconstituted PS I particles in the presence of 50 μM benzyl viologen. Although P700 mainly contributes to these two spectra, the shape and extent of the difference between these spectra (difference spectrum of the decay phase sensitive to benzyl viologen) resembles that of P430 (FeS centers) reported in PS I particles [24,25]. These results suggest that the reconstitution of VK_1 to the ether-extracted PS I particles restores the turnover of FeS centers (P430).

4. DISCUSSION

Reconstitution of 1 VK_1/P700 to the VK_1-extracted PS I particles stabilized $P700^+$ and restored the turnover of FeS centers. VK_1 stoichiometrically binds to the PS I reaction center as reported in the hexane-methanol-extracted PS I particles [9] with a dissociation constant of less than 10^{-8} M, comparable with the tight binding of ubiquinones at the Q_A site of the *Rb. sphaeroides* reaction center [23].

Although the arguments against the function of VK_1 as the primary acceptor A_1 in the PS I reaction center (i.e., the insensitivity of PS I photochemistry to UV irradiation [18,19]) still remain to be solved, it is clear that the extraction of VK_1 induces the rapid charge recombination be-

tween A_0^- and $P700^+$ as reported earlier [7–10]. However, the previously reported incomplete inhibition of the reduction of FeS centers after the extraction of VK_1 at 10–15 K [7,17] may now be understood to reflect the existence of slow electron flow between A_0 and the FeS centers at a rate that is slower than the rate of charge recombination at room temperature (30 ns) [8,9] but which may be comparable to that at low temperature (50–100 ns [8,10]). The results in the present study further show that the VK_1 reconstitution not only suppresses the charge recombination but also restores the turnover of FeS centers. A small fast submillisecond decay phase seen even after the VK_1 reconstitution may reflect some change of VK_1 function site by the ether-extraction as also suggested in [9] and may reflect a higher in situ E_m value of VK_1 in the reconstituted system.

The reconstitution restores the efficient electron flow from A_0^- to FeS centers, and then to $P700^+$, in a way seen in normal PS I [5,6,24,25]. Judging from the relatively slow rate (3–12 ms) of $P700^+$ re-reduction, the component giving the difference spectrum in fig.3 seems to be F_A and/or F_B. The F_X center, which seems to reduce $P700^+$ in about a ms half time [5,6,26], may also be functioning in mediating electrons between VK_1 and other FeS centers as in normal PS I particles, since F_X is known to be reduced by continuous illumination in this type of preparation [7]. The turnover of FeS centers, thus, gives strong evidence for the identity of A_1 as VK_1.

PS I particles as isolated contain about 2 VK_1/P700 [1,2,7–9]. However, the results in the present study as well as in those presented in [9] suggest that only one VK_1 is required for PS I primary photochemistry. This is in clear contrast to the cases in the bacterial [23] and PS II reaction centers [6,27], in which two quinones are functioning in a series as the stable primary (Q_A) and secondary (Q_B) electron acceptors. These quinones are electronically interacting with a Fe atom [6,23,28,29], which is ligated by four histidine residues [27,30]. The PS I reaction center seems to lack such nonheme iron constituents [5,6]. The results in the present study indicate that the reconstituted VK_1 has an extremely negative E_m value of lower than -500 to -700 mV and is able to reduce FeS centers. Such a low E_m value can only be attained in an apolar solvent like dimethylformamide in vitro [31], and suggests that the binding site is strongly hydrophobic. This also characterizes a unique feature of the PS I quinone binding site since menaquinone, which has a naphthoquinone skeleton similar to VK_1, when reconstituted into *Rb. sphaeroides* reaction center is estimated to exhibit a more positive E_m value of -20 to -130 mV [32]. Continued study of the PS I quinone binding site may, therefore, lead to a better understanding of the critical determinants for the function of quinone in a highly efficient charge separation in the photosynthetic reaction centers.

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