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Extraction of vitamin K-1 from Photosystem I particles by treatment with diethyl ether and its effects on the A_1^- EPR signal and System I photochemistry

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Treatment of Photosystem I particles, prepared by digitonin treatment of spinach chloroplasts, with dry diethyl ether extracted all the vitamin K-1 (2.2 molecules / P-700 in the original Photosystem I particles) and carotenoids as well as 60% of antenna chlorophylls. P-700 and FeS centers (F_X, F_A and F_B) were not extracted, and were photochemically oxidized and reduced, respectively, upon continuous illumination. This preparation showed the A_0^- EPR signal but no A_1^- signal even after long illumination at 210 or 230 K, in which condition A_1^- was accumulated in the untreated Photosystem I particles. Laser flash excitation induced only a small absorption change, corresponding to 20% of the total P-700, at 429 nm in the extracted preparation, suggesting that charge recombination took place on a submicrosecond time scale. Addition of vitamin K-1 or K-3 but not that of benzyl viologen increased the extent of the slow decay phase of $P\text{-}700^+$. The extraction also induced a new fluorescence band peaking at 692 nm, the intensity of which increased upon addition of dithionite. This fluorescence band is possibly related to the rapid charge recombination between A_0^- and $P\text{-}700^+$. These results strongly suggest that A_1 is vitamin K-1 and that it functions to mediate electron transfer between A_0 and FeS centers. Similar results were also obtained with the 'P-700 enriched particles' with 7–16 antenna chlorophyll *a* / P-700 which can be obtained by extraction of Photosystem I particles with diethyl ether containing (80% saturated) water.

Introduction

The reducing side of Photosystem I of green plants is now considered to be composed of electron acceptor molecules named A_0, A_1 and iron-sulfur centers (F_X, F_A and F_B) [1,2]. A_0, which is postulated to be the immediate electron acceptor from P-700, gives rise to a free radical type EPR signal in the $g = 2.0$ region with a line-width of about 14 gauss when reduced [3–6] and seems to be a monomeric chlorophyll peaking at 670 nm according to the photoaccumulation studies [7–9], although recent picosecond absorption spectroscopy studies [10,11] proposed chlorophyll *a* peaking at 690 nm to be primary acceptor or first intermediate. A_1, which is postulated to function between A_0 and FeS centers [2–5], also shows an EPR signal in the $g = 2.0$ region with a line-width of about 10 gauss [3–5]. Mansfield and Evans [12] recently reported a difference absorption spectrum of A_1/A_1^- in the ultraviolet and blue regions and suggested the chemical identity of A_1 to be vitamin K-1 which is known to be present in Photosystem I in an amount of about 2–3 molecules/P-

Abbreviation: PS I, Photosystem I.

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700 [13,14]. Brettel et al. [15] reported a rapid turnover of a similar ultraviolet absorbing component at 4 K.

In the present study we show that the extraction of PS I particles with diethyl ether almost completely removes vitamin K-1 and also eliminates the A_1^- EPR signal but not the signals of A_0^- and FeS centers. Effects of this treatment on the flash-induced absorbance change of P-700 were also studied. The results strongly suggest that the chemical identity of A_1 is vitamin K-1 and that vitamin K-1 functions as an electron carrier between A_0 and FeS centers and thereby stabilizes $P\text{-}700^+$. Similar results were also obtained with the 'P-700 enriched particles' which also lack vitamin K-1 and have a smaller number of antenna chlorophylls.

Materials and methods

The preparation of PS I particles, including the diethylether treatment was described previously [16]. PS I particles, prepared from digitonin-treated spinach chloroplasts, were lyophilized and then extracted twice with diethyl ether containing water as specified in the Results section. The extracted PS I particles thus obtained were solubilized in phosphate buffer (10 mM, pH 8.0) (in some cases containing 0.1% Triton X-100) by incubation for 15 min at 0–4°C. Prior to use, the supernatant was diluted about 2–20 times with appropriate buffer solutions. Temperature was kept below 10°C during all treatments and measurements to prevent damage of the samples.

Absorption spectra were measured with a Hitachi 557 dual-wavelength spectrophotometer as described previously [17]. Flash-induced absorption changes were measured with a split-beam spectrophotometer (1 μs time resolution) constructed in the National Institute for Basic Biology (NIBB). Absorption changes induced by a 15 ns (halfwidth) laser pulse at 532 nm from the second harmonic of a Nd-YAG laser (Quanta-Ray DCR20) was detected by a photomultiplier (Hamamatsu Photonics, R268) and averaged by a signal averager (50 ns/word × 4000 words, 8 bit, Autnics S121/F601) after amplification. EPR signals were measured with a Brucker EPR-200 X-band spectrometer using an Oxford Instruments ESR-900 continuous liquid helium flow cryostat or a Brucker ER 411 VT N_2 gas variable temperature unit in the Center of Analytical Instruments of NIBB [18]. Fluorescence was measured with a Hitachi 850 spectrofluorometer.

P-700 was assayed by measurements of the ferricyanide (0.5 mM)-oxidized minus ascorbate (5 mM)-reduced difference spectrum, using an extinction coefficient of 64 $mM^{-1} \cdot cm^{-1}$ for the red maximum [19]. Chlorophyll concentrations were measured by the method of Arnon [20]. Vitamin K-1 was assayed according to the method of Aoki et al. [21] with a slight modification. Lyophylized PS I particles or diethyl-ether-extracted PS I particles were dried and extracted with a 1:1 (w/w) mixture of acetone/methanol. The extract was evaporated and separated on an acid-washed alumina column (Woelum A-super I containing 8% H_2O) after dissolving in *n*-hexane. The column was eluted with a stepwise gradient mixture of *n*-hexane and diethyl ether and the vitamin K-1 containing fraction was collected under continuous monitoring of absorbance at 254 nm. A reduced minus oxidized difference absorption coefficient of 19 $mM^{-1} \cdot cm^{-1}$ at 248 nm in 90% ethanol [22] was used to determine vitamin K-1. Iron and acid-labile sulfur were determined as reported [9]. Vitamin K-1 was obtained from Sigma.

Results

Extraction of vitamin K-1 and antenna chlorophylls by ether treatment

Chemical compositions of PS I particles extracted with ether containing different amounts of water are summarized in Table I. The extraction completely eliminated vitamin K-1 and carotenoids from the PS I particles in every case. On the other hand, more chlorophylls were extracted with a higher content of water. Neither acid-labile FeS nor P-700 was extracted by the extraction. At least 70% of P-700 was photooxidizable in each preparation. There was no relation between the extent of extraction of chlorophyll and vitamin K-1.

Change of A_0^- and A_1^- EPR signals

Mansfield and Evans [5] reported that a long illumination at 200 K of PS I particles prein-

cubated with dithionite at pH 10 accumulates A_1^- and that a subsequent illumination at 215 K accumulates A_0^- in addition to A_1^-. Accumulation of A_1^- and A_0^- by illuminations at 210 or 220 K was monitored by the EPR measurement at 210 K in the untreated PS I particles (Fig. 1 upper traces). Illumination at 210 K at first induced a narrow (about 10 G peak-to-peak) signal which corresponds to A_1^- (e.g., a curve at 2 min in upper traces in Fig. 1). After longer illumination at 210 K the signal with a larger line-width (A_0^-) developed, showing small (8 G) shoulders due to P-700$^+$. The A_0^- signal became dominant after further illumination at 220 K. Time courses of the development of these two signals are shown in Fig. 2A in which the narrow (A_1^-) and broad line-width (A_0^-) components were represented by the developments of signal intensities at the magnetic fields indicated by arrows a ($A_1^- + A_0^-$) and b (A_0^-) in the upper traces of Fig. 1. In the same figure a rough estimation of the development of A_1^- signal (curve a′), calculated from curves a and b using the signal shapes of A_0^- (Fig. 1, largest trace in the ether extracted sample) and of A_1^- (Fig. 1, lower difference curve), is also shown. A_1^- (curve a or a′) developed more rapidly than A_0^-. The latter signal developed only after the accumulation of A_1^- showing a time lag at 210 K, and more significantly increased after illumination at 220 K.

Similar measurements on dry-ether-extracted PS I particles lacking vitamin K-1 showed no accumulation of A_1^- (Fig. 1, middle traces). The signal shape did not change during the course of illumination at 210 K and 220 K, indicating that only A_0^- is photoaccumulated (with small shoulders due to P-700$^+$). Difference spectra calculated between the fully illuminated preparations (i.e., untreated minus extracted preparations) clearly showed the features of A_1^- spectrum with a larger g value and a narrower line-width than those of A_0^- (lower traces in Fig. 1). Thus it is concluded that there is virtually no accumulation of A_1^- in the ether-extracted preparation. As seen in Fig. 2B the signal intensities at the two magnetic fields developed in parallel, indicating the accumulation of only A_0^- in this preparation. It should also be noted that the illumination at 210 K, which was inefficient in accumulating A_0^- in the untreated preparation, almost fully accu-

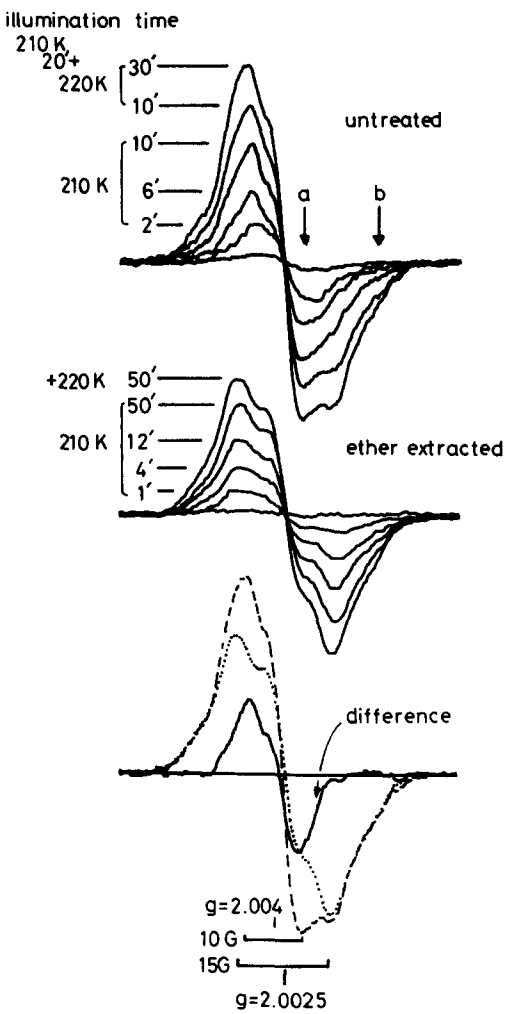

Fig. 1. Effects of dry ether extraction on the photoaccumulation of A_0^- and A_1^- EPR signals at 210 K. Upper traces, untreated PS I particles (149 chlorophylls/P-700). Middle traces, dry-ether extracted PS I particles (56 chlorophylls/P-700) lacking vitamin K-1. Lower traces, difference between the largest (with the longest illumination time) spectra of the untreated (broken line) and the ether-treated (dotted line) PS I particles in the upper and middle traces. Samples were illuminated by white light from a 150 W tungsten iodine lamp through a light guide in a N_2 gas cryostat at temperatures and lengths as indicated in the figure. Samples were dissolved in a medium containing 100 mM glycine-NaOH (pH 10) buffer and 10 mM sodium dithionite to be 0.63 μM P-700. Signals were measured at 210 K at microwave frequency and power of 9.62 GHz and 0.2 mW, respectively, modulation amplitude of 2 G, receiver gain of $5 \cdot 10^5$, scan time of 50 s and averaged for 2 times.

TABLE I

CHEMICAL COMPOSITION OF PS I PARTICLES EXTRACTED WITH DIETHYL ETHER CONTAINING DIFFERENT AMOUNTS OF WATER

n.d., not determined; Chl, chlorophyll.

H_2) content in ether (% saturation)	Chl *a* (%)	Recovery of P-700 (%)	Chl *a* (in mol per mol P-700)	Carotenoid	Vitamin K-1	Fe	S
Untreated PS I particles							
–	100	100	145	20	2.2	n.d.	n.d.
Etherextracted particles							
0	40	100	60	0	0	n.d.	n.d.
50	12	100	18	0	0	n.d.	n.d.
80	5.4	101	8	0	0	11	12.6

mulated A_0^- in the ether-extracted preparation. This again supports the view that A_1 is absent in this preparation, since reduction of A_1, if it accepts electrons from A_0^-, is required before the accumulation of A_0^-. These results strongly suggest that vitamin K-1 is the component giving rise to A_1^- signal. Such lack of A_1^- signal was apparent already in Fig. 4 of a previous study [9] on the 'P-700 enriched particles', although it was not explicitly mentioned there [9].

EPR signals of FeS centers in the ether-extracted Photosystem I particles

The extraction with diethylether seemed to have minor effects on the FeS centers. FeS contents after the extraction (Table I) were almost the same that reported for normal PS I particles [23]. Fig. 3 shows the effects of ether-extraction on the EPR spectra of F_A and F_B measured at 10 K. In every preparation illumination at 10 K reduced F_A and F_B centers. F_B seemed to be preferentially reduced

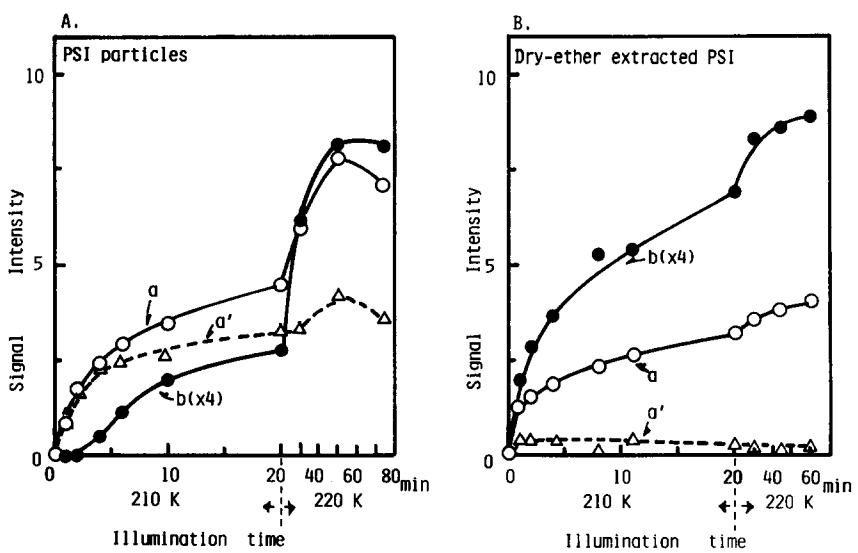

Fig. 2. Effects of ether extraction on the development of A_0^- and A_1^- signals. (A) Untreated PS I particles. (B) Dry-ether-extracted PS I particles. Intensities of EPR signals at the magnetic fields shown by arrows a and b in Fig. 1 were plotted against illumination time as curves a and b, respectively. Curve a' shows the intensity of A_1^- signal estimated from curves a and b by assuming the A_0^- and A_1^- signal shape to be that at 50 min illumination in the middle traces and the difference in the lower traces of Fig. 1, respectively.

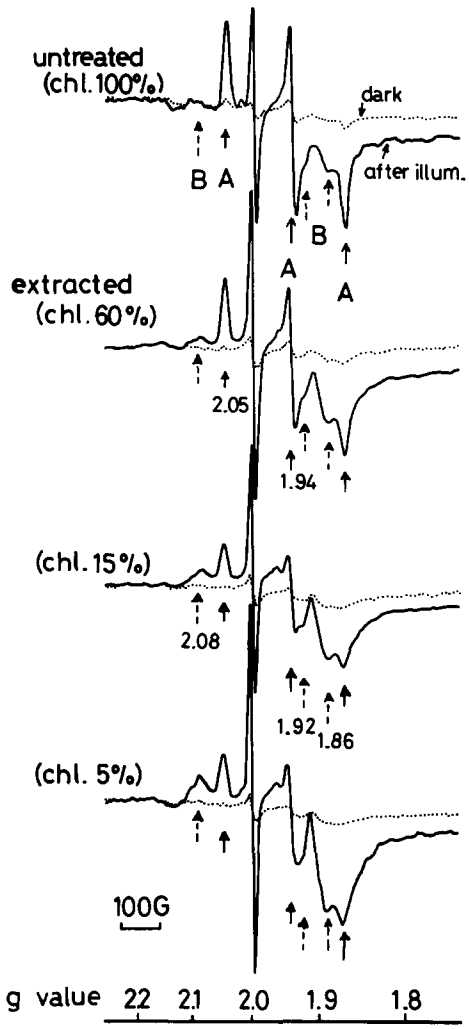

Fig. 3. Effects of ether treatment on the EPR signals of FeS centers measured at 10 K. Broken lines, frozen in the dark. Solid lines, after illumination for 3 min with a white light at 10 K. EPR signals of PS I particles extracted with diethyl ether containing various amounts of water (in 0–80% saturation) are shown (the amounts of chlorophylls remaining are shown in % of that of the original PS I particles). Concentrations of P-700 were adjusted to 27.5 μM in all the preparations. Microwave frequency and power, 9.6 GHz and 20 mW. Modulation amplitude, 10 G. Other conditions were similar to those in Fig. 1. chl., chlorophyll.

in preparations with less antenna chlorophylls. Even in the preparation with only 5% antenna chlorophylls (P-700 enriched particles), the signal intensity of photoreduced F_A (e.g., at $g = 2.05$) at 10 K was about 60% of that in the unextracted one. In the preparations with less antenna chlorophylls, however, the F_B signal (at $g = 2.08$ or at $g = 1.86$) became larger. The decrease of F_A signal was also accompanied by the appearance of $g = 1.96$ and $g = 1.99$ signals which can be reduced by illumination at room temperature (not shown; see Ref. 24), indicating that the latter components (designated as centers C and D [25] previously) are degradation products of F_A. When the samples were frozen during illumination also the F_X signal was observed, although its position was somewhat shifted depending on the extent of chlorophyll extraction [24]. These results indicate that almost all FeS centers can be reduced photochemically at 10 K and at room temperature even after the extraction of vitamin K-1 if the illumination is provided by a continuous light.

Laser-flash-induced absorption change of P-700

Flash-induced absorption changes of P-700 were studied at 429 nm at 8°C in the dry-ether-extracted PS I preparation (Fig. 4, upper traces). The intensity of laser flash was adjusted to be nearly saturating (to give absorbance change 77% of the saturated level) to avoid absorption changes due to the triplet state of antenna chlorophylls. The flash excitation induced only a small (about 1/4 of the total P-700) absorption change even when all P-700 is reduced and A_0 is oxidized before the excitation. The decay kinetics of this small absorbance change was biphasic with almost equal extent of the rapid and the slow phase, which had half times of 35 μs and more than 100 ms (which depended on the concentration of dichloroindophenol added), respectively (Fig. 4). The rapid phase may reflect the decay of triplet state of P-700 [26–29]. In the ferricyanide-oxidized preparation a smaller absorption change was detected (not shown). Re-addition of vitamin K-1 or vitamin K-3 to this preparation significantly increased the slow phase. 3 μM vitamin K-1 was sufficient for the full development of the slow phase. This corresponds to an enhancement from 20% (no addition) to 75% (with vitamin K-1) of total P-700 when the laser power was saturating. The extent of the rapid phase remained almost constant in contrast to the increase of the slow phase. A small amount of Triton X-100 (0.05%) was found to accelerate the effect of vitamin K-1. It may increase the solubility of vitamin K-1 in

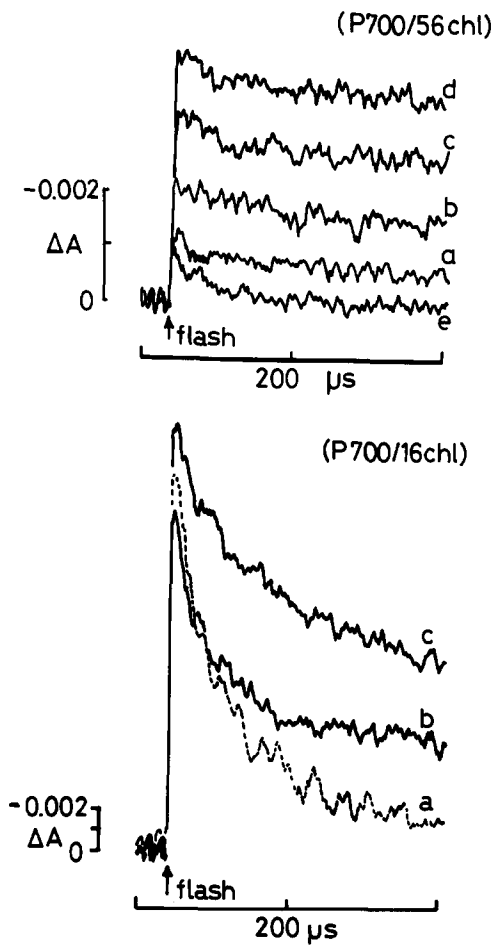

Fig. 4. Effects of re-addition of vitamin K-1 to the ether-extracted PS I particles on the flash induced absorption change at 429 nm. Upper traces: the dry-ether-extracted PS I particles (P-700/chlorophyll ratio of 1/56) at 0.17 μM P-700 concentration. (a) No addition; (b), (c) and (d) with 0.3, 1.0 and 3.0 μM vitamin K-1, respectively; (e) with 0.1 mM benzyl viologen. Lower traces: 'P-700 enriched particles' (the PS I particles extracted with 80% water-saturated ether, P-700/chlorophyll ratio of 1/16) at 1.1 μM P-700 concentration. (a) No addition; (b) about 10 min after addition of 30 μM vitamin K-3; (c) same as (b), after 1 h incubation. Samples were suspended in a medium containing 100 mM Tris-HCl buffer (pH 7.2), 5 mM sodium ascorbate, 5 μM dichloroindophenol and 0.05% Triton X-100. Each trace is an average of 256 scans with flash repetition rate of 0.5 Hz. The intensity of the laser excitation flash of 532 nm was adjusted to induce about 77% of the maximum flash-induced absorption change (0.5 mW) to avoid artifacts in the experiments in the upper traces and to be saturating (3.5 mW) in the lower traces. chl., chlorophyll.

water. Benzyl viologen, which is known to work as an efficient electron acceptor from A_0 in SDS-treated PS I preparations which are partially depleted of vitamin K-1 [29], however, did not increase the absorption change even when added at a high concentration (0.1–0.2 mM) as seen in the same figure (curve c).

Similar but a little different results were obtained in the 'P-700-enriched particles, which were prepared by extraction with wet ether (80% water-saturated) and have a P-700/chlorophyll ratio of 16 (Fig. 4, lower traces). In this preparation flash excitation (of saturating intensity) induced only a small amount of slow phase (stable $P\text{-}700^+$), 3% of that expected for the total P-700, and induced mainly a rapid decay phase with a half time of 35 μs. On addition of vitamin K-1 or K-3 the rapid phase decreased and the slow phase increased. A rather long incubation time was required to fully develop the effects of vitamin K-1 or K-3 in this preparation. After incubation for 1 h with 30 μM vitamin K-3 (trace c in the lower traces in Fig. 4), the extent of the absorbance change at 1 μs was 34% of that expected for total P-700, with a slow phase of 60%, i.e., about 20% P-700 was stabilized in microseconds time range. The rapid phase (probably triplet state) showed a difference absorption spectrum with larger band-width centering at 430 nm than that of the slow phase ($P\text{-}700^+/P\text{-}700$). Thus, in this preparation the triplet yield is higher and the amount of $P\text{-}700^+$ stabilized is lower than in the dry-ether-extracted preparation, both in the absence and presence of added quinones.

These results suggest that $P\text{-}700^+$ produced by the flash excitation is very rapidly re-reduced, in a time range shorter than the response time (1 μs) of the present measuring system, and that vitamin K_1 or K_3 works as a very efficient and specific electron acceptor to A_0^- especially in the dry-ether-extracted PS I preparation. The rather low efficiency of the added vitamin K-3 in recovering the stabilized $P\text{-}700^+$ (20%) in the P-700-enriched particles may be related to the increased triplet yield. Direct measurement of the absorption change due to the charge recombination between A_0^- and $P\text{-}700^+$ in the nonoseconds time range will be reported elsewhere (Ikegami, I., Sétif, P. and Mathis, P., unpublished data).

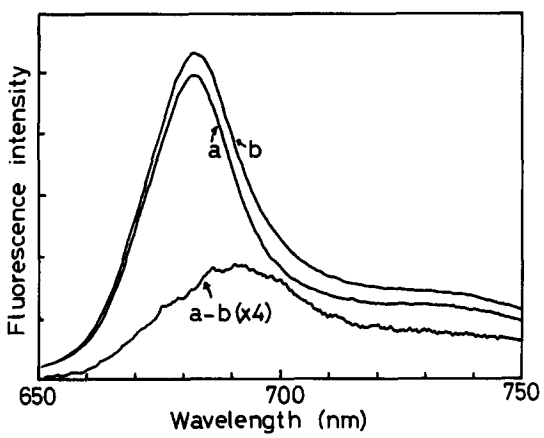

Fig. 5. Fluorescence emission spectra of the dry-ether-extracted PS I particles. (a) Ferricyanide (0.2 mM) oxidized sample; (b) dithionite (5 mM) reduced sample. Samples were suspended in a medium containing 100 mM Tris-HCl buffer (pH 7.2) at a concentration of 0.017 μM P-700. Measurements were done at 7°C, with an excitation wavelength and slit width of 440 nm and 10 nm, respectively, and with an emission slit width of 2 nm.

Fluorescence of the ether-extracted preparation

Fluorescence of the dry-ether-extracted preparation was studied at 8°C. In the presence of ferricyanide, the fluorescence emission spectrum showed a single peak at 678 nm (curve a in Fig. 5). Reduction of P-700 by ascorbate or by dithionite increased the intensity at the longer-wavelength side of this peak (curve b). Difference spectra (reduced minus oxidized) showed a peak apparently at around 692 nm. This fluorescence band was not detected in the original, untreated PS I particles (not shown) and seems to be similar to the 694 nm fluorescence band reported in the 'P-700 enriched particles' [30,31], which can be observed only when P-700 is reduced and increases further with the increase of reduced FeS centers and was therefore proposed to be emitted as a result of the recombination reaction between $P\text{-}700^+$ and A_0^- [30]. The results reported here then support our conclusion that the recombination reaction is increased by the depletion of vitamin K-1.

Discussion

Vitamin K-1 in PS I particles was completely removed by the extraction with diethyl ether. This extraction of vitamin K-1 did not correlate with the extraction of antenna chlorophylls. The extraction with diethyl ether also eliminated the EPR signal of A_1^-, accelerated the photoaccumulation of A_0^- at 210 K and enhanced the fluorescence emission which is attributed to $P\text{-}700^+A_0^-$ recombination. This suggests that A_1 is vitamin K-1.

Even when P-700 was in its reduced state only a small absorption change was detected at 429 nm in the preparation treated with dry ether. This suggests that $P\text{-}700^+$ formed by the laser flash, was reduced by back reaction with a reduced acceptor in a time range shorter than the response time of the present experimental system (1 μs). Reduction of $P\text{-}700^+$ by the reduced FeS centers is estimated to proceed with half times of longer than 250 μs from the optical measurement of P-430 [2,26–28]. A more likely cause of the reduction of $P\text{-}700^+$ in less than 1 μs may be the reaction:

$$P\text{-}700^+A_0^- \rightarrow P\text{-}700\,A_0$$

This reaction seems to proceed with a half-time of 40 ns in the P-700 enriched particles according to the recent measurements (Ikegami, I., Sétif, P. and Mathis, P., unpublished data).

That the re-addition of vitamin K-1 specifically stabilized $P\text{-}700^+$ (75% in the dry-ether-extracted PS I particles) indicates that the oxidation of A_0^- by re-added or native vitamin K_1 is fast enough (presumably subnanosecond) to prevent the charge recombination. This agrees with the estimated short life time of absorption transient with a peak at 690 nm reported recently [10,11]. On the other hand, the observation that, even in the absence of vitamin K-1 20% of $P\text{-}700^+$ remained oxidized in the microseconds time range suggests that the reoxidation of A_0^-, presumably by F_X, is only about 5 times slower than the recombination reaction, i.e., completes in a few hundred ns. This rather rapid reaction time in the absence of vitamin K-1 suggests that the distance between A_0 and F_X is quite short.

The recombination between $P\text{-}700^+$ and A_0^- via a pathway giving rise to the P-700 triplet state has been estimated to take 10–200 ns [2]. That nevertheless only a small triplet yield was observed in the dry-ether-extracted preparation (a small 35 μs

phase in the 429 nm absorption change), suggests that the efficiency of triplet formation is not large even in the absence of A_1. In the 'P-700 enriched particles' some changes in the structure of reaction center proteins or chlorophyll organization may increase the triplet yield and also may decrease the effectiveness of re-added quinones.

The time constants for the reoxidation of A_0^- in the presence and absence of A_1 estimated here seem to explain the rapid and efficient electron transfer on the reducing side of native PS I, although there remains some ambiguity concerning the molecular species of A_0 itself [1,2,10,11].

In the absence of vitamin K-1, photooxidation of P-700 and photoreduction of FeS centers were still detected at 10 K and at room temperature under continuous illumination. This result does not conflict with the idea that vitamin K-1 is A_1, since a long continuous illumination can reveal reactions of rather low efficiency (i.e., competing with a high recombination rate). The reduction of FeS centers still observed in the absence of vitamin K_1 most likely is due to direct electron transfer between A_0 and FeS centers (probably F_X). The high rate of this reaction at 8 °C and even at 10 K suggest a short distance between F_X and A_0. These molecules are probably in the same PS I core proteins [23].

The increase of the charge recombination after the extraction of vitamin K-1 may be correlated with the appearance of a new 692 nm fluorescence band, which depends on redox state of P-700. A change of the fluorescence yield upon the extraction of the primary acceptor quinone has also been reported for the bacterial reaction center complex [32]. If the fluorescence is produced in the recombination reaction between A_0^- and P-700$^+$, then its life time should also be related to the reaction. Precise determination of the life time of the 692–694 nm fluorescence band is required. The fluorescence life time previously measured in the 'P-700 enriched particles' (6 ns) [32] seems to require re-interpretation from another aspect.

The results in the present study show that vitamin K-1 probably acts as the electron acceptor called A_1 in Photosystem I, and that it suppresses the recombination reaction between A_0^- and P-700$^+$ by functioning as an electron mediator between A_0 and FeS centers.

Acknowledgments

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References

1 Mathis, P. (1986) Photosynth. Res. 8, 97–111
2 Rutherford, A.W. and Heathcote, P. (1985) Photosynth. Res. 6, 295–316
3 Bonnerjea, J. and Evans, M.C.W. (1982) FEBS Lett. 148, 313–316
4 Gast, P., Swarthoff, T., Ebskamp, F.C.R. and Hoff, A.J. (1983) Biochim. Biophys. Acta 722, 168–175
5 Mansfield, R.W. and Evans, M.C.W. (1985) FEBS Lett. 190, 237–241
6 Heathcote, P., Timofeev, K.N. and Evans, M.C.W. (1979) FEBS Lett. 101, 101–105
7 Shuvalov, V.A., Dolan, E. and Ke, B. (1979) Proc. Natl. Acad. Sci. USA 76, 770–773
8 Swarthoff, T., Gast, P., Amesz, J. and Buisman, H.P. (1982) FEBS Lett. 146, 129–132
9 Ikegami, I. and Ke, B. (1984) Biochim. Biophys. Acta 764, 70–79
10 Nuijs, A.M., Shuvalov, V.A., Van Gorkom, H.J., Plijter, J.J. and Duysens L.N.M. (1986) Biochim. Biophys. Acta 850, 310–318
11 Shuvalov, V.A., Nuijs, A.M., Van Gorkom, H.J., Smit, H.W.J. and Duysens, L.N.M. (1986) Biochim. Biophys. Acta 850, 319–323
12 Mansfield, R.W. and Evans, M.C.W. (1986) FEBS Lett. 203, 225–229
13 Takahashi, Y., Hirota, K. and Katoh, S. (1985) Photosynth, Res. 6, 183–192
14 Schroeder, H.E. and Lockau, W. (1986) FEBS Lett. 199, 23–27
15 Brettel, P., Sétif, P. and Mathis, P. (1986) FEBS Lett. 203, 220–224
16 Ikegami, I. and Katoh, S. (1975) Biochim. Biophys. Acta 376, 588–592
17 Ikegami, I. and Itoh, S. (1986) Biochim. Biophys. Acta 851, 75–85
18 Itoh, S., Tang, X.S. and Satoh, K. (1986) FEBS Lett. 205, 275–281
19 Hiyama, T. and Ke, B. (1972) Biochim. Biophys. Acta 267, 160–171
20 Arnon, D.I. (1949) Plant Physiol. 24, 1–15
21 Aoki, M., Hirano, M., Takahashi, Y. and Katoh, S. (1983) Plant Cell Physiol. 24, 512–525

22 Barr, R. and Crane, F.L. (1971) Methods Enzymol. 23, 372–408
23 Sakurai, H. and San Pietro (1985) J. Biochem. 98, 69–76
24 Ikegami, I. and Itoh, S. (1987) Biochim. Biophys. Acta 893, 517–523
25 Hiyama, T., Murakami, A., Itoh, S., Fujita, Y. and Sakurai, H. (1985) J. Biochem. 97, 89–95
26 Sauer, K., Acker, S., Mathis, P. and Van Best, J. (1978) Biochim. Biophys. Acta 503, 120–134
27 Koike, H. and Katoh, S. (1982) Photochem. Photobiol. 35, 527–531
28 Nechushtai, R., Nelson, N., Gonen, O. and Lavano, H. (1985) Biochim. Biophys. Acta 807, 35–43
29 Takahashi, Y. and Katoh, S. (1984) Plant Cell Physiol. 25, 785–794
30 Ikegami, I. and Ke, B. (1984) Biochem. Biophys. Acta 764, 80–85
31 Ikegami, I. (1976) Biochim. Biophys. Acta 449, 245–248
32 Woodbury, N.W., Parson, W.W., Gunner, M.R., Prince, R.C. and Dutton, P.L. (1986) Biochim. Biophys. Acta 851, 6–22
33 Kamogawa, K., Morris, J.M., Takagi, Y., Nakashima, N., Yoshihara, K. and Ikegami, I. (1983) Photochem. Photobiol. 37, 207–213