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Contribution of Vitamin K_1 to the Electron Spin Polarization in Spinach Photosystem I†

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ABSTRACT: The electron spin polarized (ESP) electron paramagnetic resonance (EPR) signal observed in spinach photosystem I (PSI) particles was examined in preparations depleted of vitamin K_1 by solvent extraction and following biological reconstitution by the quinone. The ESP EPR signal was not detected in the solvent-extracted PSI sample but was restored upon reconstitution with either protonated or deuterated vitamin K_1 under conditions that also restored electron transfer to the terminal PSI acceptors. Reconstitution using deuterated vitamin K_1 resulted in a line narrowing of the ESP EPR signal, supporting the conclusion that the ESP EPR signals in the reconstituted samples arise from a radical pair consisting of the oxidized PSI primary donor, P_{700}^+, and reduced vitamin K_1.

A characteristic electron spin polarized (ESP)[1] electron paramagnetic resonance (EPR) signal was first observed in plant photosystem I (PSI) a number years ago (Thurnauer et al., 1979; McIntosh et al., 1979; McCracken et al., 1982; Gast et al., 1983). Yet, the identity of this signal is uncertain. Evidence suggests that the signal is due to $P_{700}^+A_1^-$, where P_{700}^+ is the oxidized primary chlorophyll donor of PSI and A_1^- is believed to be a PSI acceptor in the electron transport chain $P_{700}A_0A_1F_xF_aF_b$. Acceptor A_0 is probably a chlorophyll species, A_1 is believed to be a quinone-like molecule, and F_x, F_a, and F_b are iron–sulfur centers (Golbeck, 1987; Mathis &

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[1] Abbreviations: ESP, electron spin polarization; EPR, electron paramagnetic resonance; PSI, photosystem I; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; HPLC, high-performance liquid chromatography.

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Rutherford, 1987). Furthermore, the ESP EPR signal observed in PSI is similar to the ESP EPR signal observed in iron-removed reaction centers (RC) found in the photosynthetic bacterium *Rhodobacter sphaeroides* (Gast, 1982; DeGroot et al., 1984; Pedersen et al., 1987). The bacterial ESP EPR signal has been assigned to the $P_{870}^{+}Q^{-}$ radical pair, where P_{870}^{+} is the oxidized special pair primary donor, a bacteriochlorophyll dimer, and Q^{-} is a reduced ubiquinone molecule (Gast, 1982; DeGroot et al., 1984; Petersen et al., 1987).

The role of vitamin K_1 (phylloquinone) found in PSI and its possible identity as the A_1 acceptor have been investigated both by organic solvent extraction/reconstitution of vitamin K_1 and by photodestruction experiments followed by optical and EPR spectroscopic measurements (Malkin, 1986; Lockau et al., 1986; Palace et al., 1987; Itoh et al., 1987; Ziegler et al., 1987; Setif et al., 1987; Mansfield et al., 1987; Ikegami et al., 1987; Biggins & Mathis, 1988; Biggins, 1990; Biggins et al., 1989). In this paper, we examine the effect of vitamin K_1 extraction and reconstitution from spinach PSI particles on the ESP EPR signal.

Materials and Methods

Spinach PSI particles were isolated, purified, and lyophilized, and vitamin K_1 was extracted with a hexane–methanol solvent mixture by procedures described previously (Setif et al., 1987; Biggins & Mathis, 1988; Biggins, 1990). The untreated (control) and extracted PSI particles were rehydrated in Tricine buffer (pH = 7.5) with 0.2% Triton X-100 and centrifuged to remove insoluble materials (Setif et al., 1987; Biggins & Mathis, 1988; Biggins, 1990).

Protonated vitamin K_1 was obtained from Aldrich Chemical Co. and used without further purification. Perdeuterated vitamin K_1 was extracted from the cyanobacterium *Synechococcus lividis* (99.6% deuterated) and purified with HPLC. Purity was checked by absorption spectroscopy and mass spectroscopy (Gast et al., 1985; Barry et al., 1988). Reconstitution of extracted PSI was accomplished by addition of the desired quinone directly to the PSI preparation (50 μM vitamin K_1 final concentration) (Biggins & Mathis, 1988; Biggins, 1990).

EPR X-band measurements were done with a Varian E-9 spectrometer equipped with an Air Products low-temperature accessory. A 300-W xenon arc lamp was modulated at 500 Hz, and ESP EPR signals were collected with a phase-sensitive detection system referenced to the modulated light source (Levanon, 1979; Feezel et al., 1989). The sample was dark adapted and then frozen in liquid nitrogen. A preliminary scan was run on the dark sample to check for spurious signals. The g-values were calibrated by comparison to a powder DPPH sample (g = 2.0037). Phase angles were adjusted to maximize the ESP EPR signals. All spectra were recorded at $\approx$10 K.

Results

Continuous Light. Photoinduced EPR signals of P_{700}^{+} obtained by continuous illumination of the control, extracted, and reconstituted PSI particles were all very similar with g-values around 2.0025 and line widths of about 7.5 G. All signals were found to be about 10% reversible. F_a^{-} and F_b^{-} signals were also observed in control and extracted PSI particles upon continuous illumination, in agreement with previous work (Setif et al., 1987).

Light Modulation. The transient ESP EPR signals in the region of g = 2.00 of the control and the extracted PSI particles are shown in Figure 1. The PSI particles reconstituted with either protonated or deuterated vitamin K_1 are presented

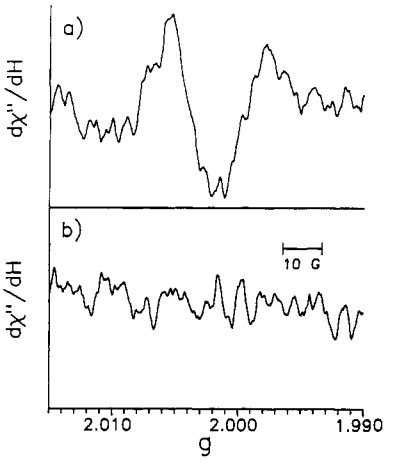

FIGURE 1: X-Band ESP EPR signals of spinach PSI particles collected at 500-Hz light modulation, 0.5-mW microwave power, and 2-G modulation amplitude (100-kHz field modulation). (a) The control sample; (b) the vitamin K_1 extracted sample (4-G modulation amplitude).

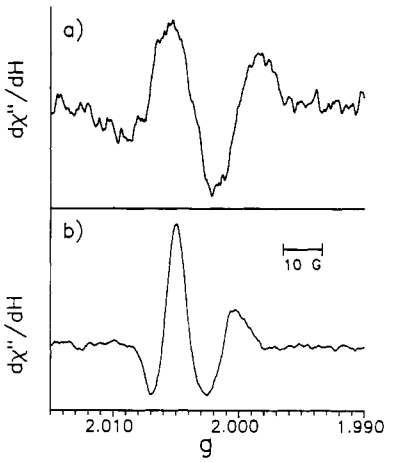

FIGURE 2: X-Band ESP EPR signals of vitamin K_1 reconstituted spinach PSI particles collected at 500-Hz light modulation, 0.5-mW microwave power, and 2-G modulation amplitude (100-kHz field modulation). (a) Protonated vitamin K_1; (b) deuterated vitamin K_1 (1.5-G modulation amplitude).

in Figure 2. With the exception of the extracted case, all samples exhibited a characteristic emission/absorption/emission (E/A/E) polarization pattern. Reconstitution using protonated vitamin K_1 did not affect line width, but deuterated vitamin K_1 reconstitution reduced low-field line width by a factor of 1.6. The line narrowing in the latter case is probably responsible for the observed g-value shifts. Signal intensity increased dramatically from the protonated to the deuterated reconstituted samples. Signal intensity was strongly dependent upon the detection phase (at 500-Hz light modulation) in all cases except for the control sample. In the latter, the signal intensity was only weakly phase dependent.

Discussion

No discernible differences exist in the appearance of the signals due to P_{700}^{+} produced by continuous illumination of the control, extracted, and reconstituted samples. Our observation of the light-induced signals of F_a^{-} and F_b^{-} in both the control and extracted samples agrees with previous reports in which low-temperature electron transfer proceeds to the terminal FeS centers with or without vitamin K_1 present in spinach PSI particles (Setif et al., 1987). However, at room temperature complete extraction of vitamin K_1 inhibits the electron transfer prior to the FeS centers but is restored after

vitamin K_1 reconstitution (Biggins & Mathis, 1988; Biggins, 1990; Itoh & Iwaki, 1989).

In the control PSI sample (Figure 1a) we observe a "normal" ESP EPR signal with the usual E/A/E pattern (Thurnauer et al., 1979; McIntosh et al., 1979; McCracken et al., 1982; Gast et al., 1983). In the extracted PSI sample with approximately the same P_{700} concentration as in the control sample, we did not observe any ESP EPR signal (Figure 1b), even at much higher sensitivity and field modulation amplitude than employed with the control. In the protonated vitamin K_1 reconstituted sample (Figure 2a), the ESP EPR signal is restored with the same E/A/E pattern and *g*-values as in the control. These data indicate that the presence of vitamin K_1 was required for the spinach PSI RC to exhibit the ESP EPR signal. Moreover, in the sample reconstituted with deuterated vitamin K_1 (Figure 2b), the line-width narrowing of part of the ESP EPR signal indicated that vitamin K_1 was not only required for spinach PSI to exhibit ESP but directly contributed to the signal.

The ESP EPR signal in PSI has been assigned to a radical pair. One partner is P_{700}^+, which contributes to the high-field portion of the spectrum (Thurnauer & Clark, 1984). The other partner is a quinone-like species (Thurnauer & Gast, 1985). We have established here that, in the reconstituted sample, the quinone is vitamin K_1, and it contributes primarily to the low-field portion of the spectrum.

The change in light modulation phase behavior between the control and reconstituted samples suggests that the extraction/reconstitution procedures perturb the temporal properties of the PSI RC. Kinetic behavior is not readily analyzed with our light modulation experiments; we are addressing it with more direct time-resolved EPR measurements on these systems. Possibly vitamin K_1 acts as an exogenous acceptor in the reconstituted sample. However, recent room temperature results on $NADP^+$ photoreduction (Biggins & Mathis, 1988) and P_{430} turnover (Biggins, 1990) demonstrate that vitamin K_1 is functionally incorporated into the PSI RC (Biggins et al., 1989). In conclusion, our results add to the growing evidence that the ESP EPR signal in spinach PSI can be assigned to a P_{700}^+–vitamin K_1^- radical pair.

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