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KINETICS OF EPR SIGNAL II_{vf} IN CHLOROPLAST PHOTOSYSTEM II

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The risetime of EPR signal II_{vf} (S II_{vf}) has been measured in oxygen-evolving Photosystem II particles from spinach chloroplasts at pH 6.0. The EPR signal shows an instrument-limited rise upon induction ($t_{1/2} \leqslant 3$ μs). These data are consistent with a model where the species Z responsible for S II_{vf} is the immediate electron donor to P-680$^+$ in spinach chloroplasts. A new, faster decay component of S II_{vf} has also been detected in these experiments.

Z is a plastoquinone species [1] which acts as an electron carrier on the oxidizing side of PS II. The oxidized form of Z, probably a semiquinone cation [2], gives rise to EPR Signal II (S II). A fast kinetic component of the signal, designated Signal II_{vf}, is involved in physiological electron transfer between the oxygen evolving enzyme and the PS II reaction center (P-680) [3–5]. When oxygen evolution is blocked, the normal source of electrons for Z^+ is destroyed, slowing the decay kinetics of S-II. Under these conditions the kinetic component decaying in 10 ms–1 s is referred to as Signal II_f [3,4]. S II_f has been shown by detailed kinetic studies to reflect donation by Z directly to the oxidized primary donor P-680$^+$ [6].

In oxygen-evolving chloroplasts and in *Chlorella pyrenoidosa,* the re-reduction of P-680$^+$ occurs in the submicrosecond range [7,8]. However, an induction time of S II_{vf} in chloroplasts was previously measured to be 20 ± 10 μs [9]. We now find that a more sensitive measurement of the rise of this signal in O_2-evolving PS II particles shows an instrument-limited rise ($t_{1/2} \leqslant 3$ μs). Thus, there is no longer any discrepancy between the P-680$^+$ reduction kinetics and S II_{vf} rise, allowing Z to be a likely candidate for the immediate donor to P-680$^+$.

Samples used were similar to other O_2-evolving PS II preparations reported in the literature [10,11]. Spinach leaves were blended for 10 s in 0.4 M sucrose/50 mM Mes (pH 6.0)/10 mM NaCl/5 mM $MgCl_2$ and filtered through 8 layers of cheesecloth. This sample was centrifuged at 7000 rpm for 10 min in a Sorvall SS34 rotor. The resulting pellet was resuspended in 50 mM Mes (pH 6.0)/10 mM NaCl/5 mM $MgCl_2$/1 mM ascorbate. The suspension was accelerated in the centrifuge to 3000 rpm, which was immediately shut off, to pellet cellular debris. The supernatant was treated with 25 mg Triton X-100 per mg chlorophyll at a sample concentration of 2 mg Chl per ml and immediately subjected to low-speed centrifugation at 3000 rpm for 4 min (SS34 rotor). The resulting supernatant was centrifuged at 18 500 rpm for 10 min. After discarding the supernatant the pellet was resuspended to 2 mg Chl/ml in 50 mM Mes (pH 6.0)/10 mM NaCl/5 mM $MgCl_2$, treated with 5 mg Triton X-100 per mg chloro-

Abbreviations: Mes, 4-morpholineethanesulfonic acid; Chl, chlorophyll; PS II, Photosystem II; S II, EPR Signal II.

phyll, spun at 3000 rpm for 5 min and the supernatant centrifuged at 18 500 rpm for 10 min. The resulting pellet was washed in 50 mM Mes (pH 6.0) and 10 mM NaCl and pelleted at 18 500 rpm for 10 min. This final pellet was then resuspended in 50 mM Mes (pH 6.0) and 10 mM NaCl to a chlorophyll concentration of 3.0 mg per ml. Potassium ferricyanide (1 mM) and ferrocyanide (1 mM) were added as exogenous electron carriers and for redox buffering. The sample was kept on ice until the measurement began. The samples exhibited O_2 evolution rates of 200–300 μM O_2/mg Chl per h initially, which decayed 10–25% during the experiment. During the measurements the samples were flowed through a Scanlon EPR flat cell at a rate of 0.4 ml min^{-1} by a Gilson minipuls II peristaltic pump and then back into an ice-cooled reservoir. At a 2 Hz flash frequency this gives the sample 15 flashes while it is in the laser beam. The temperature in the flat cell was 10 ± 2°C as measured by a Fluke 2100 A digital thermometer using a copper-constantan thermocouple. The thermocouple was located inside the flow system at the exit of the flat cell.

An X-band field-swept spectrum of the sample was taken before the experiment began. The field was then set to $g = 2.010$ (low-field hyperfine maximum of S II), and a 1000 pass kinetic trace of the decay of S II_{vf} was acquired. The sample was then used to collect S II_{vf} risetime data, which were stored after 7500 passes and again after 15 000 passes. After the risetime data were stored, a 2000 pass decay trace at $g = 2.010$ was collected. These final decay data were taken to check for the appearance of S II_f resulting from deactivation of the samples. In all cases, the final decay trace was not less than 90% S II_{vf}.

A Varian E-109 EPR spectrometer modified for 1 MHz field modulation at a modulation amplitude of 4.2 G was used for the measurements. The instrument response time was about 2 μs [12]. When field-swept spectra were taken, an output low-pass RC filter of 0.3 s was used, and the data were digitized at 36 ms/channel. When S II_{vf} decay traces were collected, an output low-pass filter of 300 μs was used, and the data were digitized at 20 μs/channel. While collecting the risetime data, the output was filtered by a 0.5 μs low-pass filter, and the data were digitized at 200 ns/channel. In all cases the output was fed into a Nicolet Explorer IIIA digital oscilloscope, and 4096 channels of data were collected. The data were accumulated in a laboratory-built signal averager. The signal-averaged trace or spectrum was sent upon completion to a VAX 11-780 computer for data analysis and display.

Light pulses were provided by a modified flashlamp-pumped dye laser (Phase-R Corp. DL-1400) which delivered pulse widths of 0.3–0.5 μs FWHM. Energies of 3–5 mJ/pulse were delivered to the sample. Rhodamine 640 (Exciton, Dayton, OH, U.S.A.) in methanol was used as the dye. A repetition rate of 2 Hz was used for all kinetic traces.

The sum of 67 500 traces can be seen in Fig. 1. On the left is the full kinetic trace and on the right is an expanded time-scale for a clearer view of the rise kinetics. As can be seen in the figure, the rise of S II_{vf} is instrument-limited ($t_{1/2} \leq 3$ μs). The decay of the signal was fit in the manner described previously [6] and is shown in Fig. 2. A clearly biexponential decay is observed with $t_{1/2} = 50 \pm 25$ μs and 0.9 ± 0.4 ms. The 50 μs decay component is 60 ± 15% of the amplitude. The other 40 ± 15% is due to the 0.9 ms component.

During our control experiments decay of S II_{vf} in chloroplasts and PS II particles at pH 6.0 and

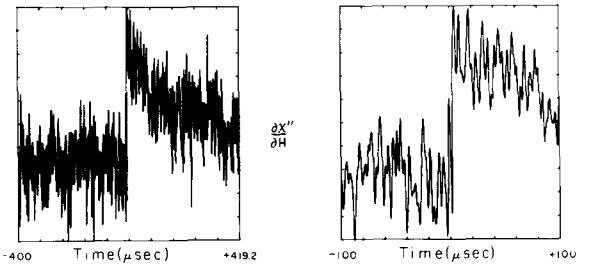


Fig. 1. EPR S II_{vf} kinetics with 2 μs time resolution. (A) Full scan, 820 μs; (B) expanded data, 200 μs. Flowing sample contained 3 mg Chl per ml, 1 mM potassium ferri/ferrocyanide, 50 mM Mes (pH 6.0), 10 mM NaCl; $T = 8$–12°C. Varian E 109 spectrometer: 1 MHz field modulation, amplitude, 4.2 G; response time, 2 μs; microwave power, 100 mW. Laser excitation intensity, 3–5 mJ/pulse at sample; 15–20 flashes per sample volume under flow; frequency, 2 Hz. Total flashes, 67 500; sample changed after each 15 000 flashes showed at least 90% S II_{vf} at $g = 2.010$.

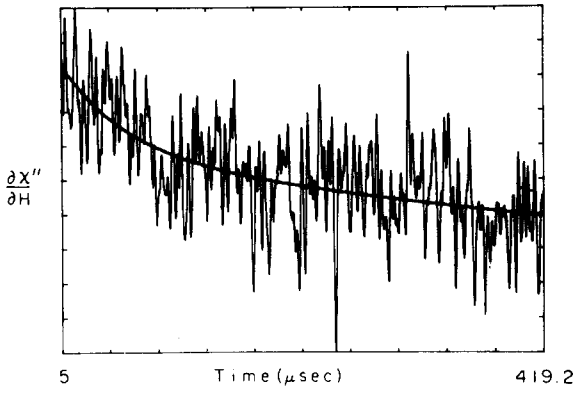


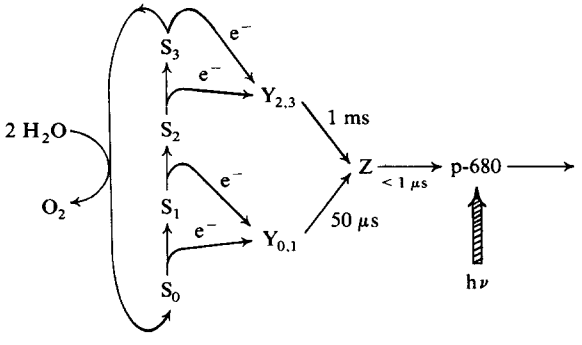
Fig. 2. Least squares fit to the decay data from Fig. 1. The biexponential decay shows $t_{1/2} = 50 \pm 25$ μs and 0.9 ± 0.4 ms decays terminating at a prerecorded baseline. Instrumental and sample conditions given in Fig. 1.

$T = 10 \pm 2$°C were measured using 100 kHz field modulation. Both the particles and the chloroplasts had decay half-times of 0.9 ± 0.3 ms, in agreement with the data collected using the 1 MHz field modulation system. The 50 μs decay component was not visible in the control experiment due to the 100 μs response time of the 100 kHz system.

A submillisecond decay component of S II_{vf} was postulated in earlier work on the flash number dependence of S II_{vf} decay [5]. In that study, decay half-times of 400 μs following the second flash, 1 ms after the third flash and only small spikes on the first and fourth flashes were seen. If $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_2$ feed electrons to Z^+ with a halftime of 50 μs and $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_0$ feed electrons to Z^+ with a half-time of 1 ms, we expect to observe biexponential decay where the sample is present in the flat cell during 15 flashes. The 400 ms halftime on the second flash would be due to a combination of a 1 ms decay and the instrument-limited spike. Experiments are being developed to test these assignments.

In our control experiments we find that our PS II particles are more stable in O_2 evolution than are chloroplasts. Even with this increased stability, cooling the sample and changing samples every 15 000 flashes was necessary. Because the S II_{vf} risetime reported in [9] was collected on a 5 ml sample for 20 000 kinetic traces at room temperature, it seems likely that much of the data collected was measuring the risetime of S II_f. Although the chloroplasts were in a pH 7.5 buffer, the thylakoid membrane's proton gradient could set the pH inside the thylakoid membrane, where the O_2-evolving system is located, to between 5.5 and 6.0, where the risetime of S II_f would be 20 μs [6,13].

The instrument-limited risetime of S II_{vf} is consistent with a model where the plastoquinone species is the immediate electron donor to the reaction center, as it is in tris-washed samples [6]. All of these data combined give rise to a model of the donor side of PS II shown below, where the plastoquinone species giving rise to S II_{vf} is the physiological electron transfer component Z donating directly to P-680. In this model, S_0–S_3 represent Kok's four-state model for the O_2-evolving enzyme [14]. Y represents an electron carrier between the enzyme and the quinone responsible for S II_{vf}.



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