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RISE TIME OF EPR SIGNAL II_{vf} IN CHLOROPLAST PHOTOSYSTEM II

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Summary

The rise time of the photoinduced, reversible EPR Signal II_{vf} in spinach chloroplasts is found using flash excitation to be 20 ± 10 μs. The results are interpreted as evidence that the Signal II_{vf} radical is an electron carrier on the donor side of Photosystem II, but probably does not result from the first donor to $P680^+$.

A new photosynthetic EPR signal, called Signal II_{vf}, has been observed recently and assigned to the physiological donor to $P680^+$ at room temperature [1—4]. The decay kinetics and inhibitor response of the signal were the primary basis of this assignment. The formation kinetics were not resolved in earlier work owing to the inadequate response time of the instrument. A measurement of the rise time of Signal II_{vf} is of interest in the assessment of its role in electron transport in Photosystem II. In this communication we report the formation kinetics of Signal II_{vf} in spinach chloroplasts.

Flash kinetic EPR measurements were made essentially as previously described [2], except that the Varian E-3 EPR instrument was modified for 1 MHz magnetic field modulation as described by Smith et al. [5]. The instrument time constant was nominally set at 10 μs. Since the time constant and the 10 μs flash duration are comparable to the rise kinetics expected for Signal II_{vf} it is important to demonstrate the overall response time of the system for a signal known to have a fast rise. This is most clearly shown by the formation kinetics of the EPR resonance called Signal I, associated with P700 oxidation, which is known to occur faster than 2 μs [6]. Fig. 1A shows the rise time under our instrumental conditions of the EPR signal at a field value corresponding to Signal I. A first order plot gives an apparent $t_{1/2}$ of 5 μs, which is indicative of the limit of the overall instrument response time. The Signal II_{vf} rise in the

Abbreviation: HEPES, *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulphonic acid.

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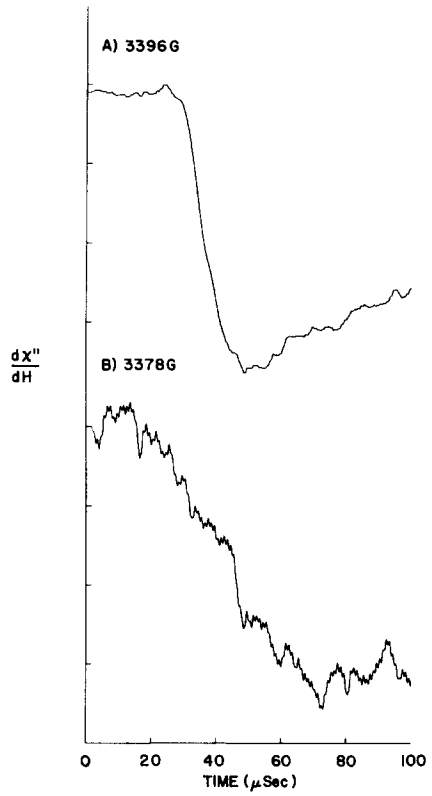

Fig. 1. A. Rise kinetics of EPR signal at a field value corresponding to Signal I in spinach chloroplasts at room temperature; monitored at the high field maximum at 3396 Gauss. B. Rise kinetics of Signal II_{vf} monitored at the low field maximum at 3378 Gauss. 10-μs xenon flashes were given at the rate of 2/s. The trace in A is the average of 3000 events, while that in B is the average of 20 000 events. Microwave power, 25 mW in A and 50 mW in B. Modulation amplitude, 4 Gauss; microwave frequency, 9.525 GHz. Chlorophyll content, 6.7 mg/ml. The chloroplast solution contained $4 \cdot 10^{-3}$ M NADP, 80 μg/ml ferredoxin, and $2 \cdot 10^{-4}$ M EDTA in 0.4 M sucrose, 0.05 M HEPES, pH 7.6, and 0.01 M NaCl. A single 5-ml sample was flowed continuously through the EPR flat cell at 0.25 ml/min. The vertical scale in B is 1.7 times expanded relative to that in A. The coupling of microwaves into the cavity was reversed between A and B so that the direction of the change would be the same in both cases. Control experiments showed no effect of cavity coupling or sample aging on rise kinetics.

same sample, shown in Fig. 1B, is calculated from a first order plot to be 20 ± 10 μs.

For an accurate determination of rise kinetics the excitation source should be very short, and the decay of the response should be slow compared to the rise. In this experiment the 10-μs flashes and the possibility of undetected fast decay components make both of these sources of error non-negligible, so the 20 μs value for $t_{½}$ for the Signal II_{vf} rise is an approximate number.

Some ambiguity has existed concerning whether Signal II_{vf} might be identical to X-320, a species thought to be the primary acceptor of Photosystem II [7,8]. The difficulty stems from the fact that the two components

have very similar decay times, about 600 μs in untreated chloroplasts. However, the decay kinetics of X-320 are not significantly changed by Tris-washing [8], a procedure which slows the Signal II_{vf} decay by as much as 1000 fold. Also, X-320 rises in less than 1 μs [8], and we now find that Signal II_{vf} is slower. These results are most compatible with an assignment of Signal II_{vf} on the donor side of Photosystem II and X-320 on the acceptor side, in agreement with previous work.

Recent fluorescence [9,10] and absorption [11,12] measurements on Photosystem II have suggested that rereduction of $P680^+$ occurs in less than 1 μs, considerably faster than the 35 μs time reported by Gläser et al. [13]. If $P680^+$ rereduction is this rapid, then Signal II_{vf} must arise from a species that is farther from the reaction center than previously thought. These experiments suggest that the unidentified component Z is located between P680 and Signal II_{vf}. The rise time of Z should be less than 1 μs and the decay should have $t_{1/2}$ = 20 μs. We can describe electron flow on the donor side of Photosystem II by the scheme shown below.

$$\text{Z P680 Q} \xrightarrow{h\nu} \text{Z P680}^+ \text{Q}^-$$

$$\downarrow\ <1\ \mu s \qquad \text{(Refs. 9—12)}$$

$$\text{Z}^+ \text{P680 Q}^-$$

$$\downarrow\ 20\ \mu s \qquad \text{(This work)}$$

$$\text{Sig II}_{vf}{}^+ \text{ Z P680 Q}^-$$

$$\downarrow\ \leqslant 0.1\text{—}1\text{ms} \qquad \text{(Refs. 2 and 3)}$$

$$\text{S}^+ \text{ Sig II}_{vf} \text{ Z P680 Q}^-$$

In this scheme the species responsible for Signal II_{vf} lies between the water-splitting enzyme S, and Z, the secondary electron donor to Photosystem II.

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