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Publication Date
1976-10-01
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October 1976

Prepared for the U. S. Energy Research and Development Administration under Contract W-7405-ENG-48

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RISE TIME OF EPR SIGNAL II\textsubscript{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⁺.

Abbreviations: HEPES, N-2-hydroxyethyl piperazine-N'2-ethanesulphonic acid.
A new photosynthetic EPR signal, called Signal II\textsubscript{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\textsubscript{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\textsubscript{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 \textit{et al.} (5). The instrument time constant was nominally set at 10 \mu s. Since the time constant and the 10 \mu s flash duration are comparable to the rise kinetics expected for Signal II\textsubscript{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 \mu s. (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 \textit{t}_{1/2} of 5 \mu s, which is indicative of the limit of the overall instrument response time. The Signal II\textsubscript{vf} rise in the same sample, shown in Fig 1b, is calculated from a first order plot to be 20 \pm 10 \mu s.

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 \mu s flashes and the possibility of
undetected fast decay components make both of these sources of error non-negligible, so the 20 μs value for $t_{1/2}$ 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 the Photosystem II by the scheme shown below.
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.

ACKNOWLEDGEMENT

This work was supported by the U. S. Energy Research and Development Administration.
REFERENCES


FIGURE LEGEND

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/sec. 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 x 10⁻³M NADP, 80 μg/ml ferredoxin, and 2 x 10⁻⁴M EDTA in 0.4M sucrose, 0.05M HEPES, pH 7.6, and 0.01M NaCl. A single 5 ml sample was flowed continuously through the EPR flat cell at .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.
This report was done with support from the United States Energy Research and Development Administration. Any conclusions or opinions expressed in this report represent solely those of the author(s) and not necessarily those of The Regents of the University of California, the Lawrence Berkeley Laboratory or the United States Energy Research and Development Administration.