REACTION KINETICS FOR POSITIVE CHARGE ACCUMULATION ON THE WATER SIDE OF CHLOROPLAST PHOTOSYSTEM II

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1. Introduction

Recent experiments in our laboratory [1–3] have identified an e.p.r. component, Signal IIvf, as arising from the oxidized donor to P680, Z^+. Following a saturating flash the formation of Signal IIvf, corresponding to the oxidation of Z, occurs within 100 μsec. The decay, which is attributed to the rereduction of Z^+ by the S states [4], occurs with a halftime of approx. 700 μs. However, these parameters were determined under steady state conditions, and therefore this 700 μsec decay for Signal IIvf reflects the average halftime for the reaction $Z^+ + S_n \rightarrow Z + S_{n+1}$ where n = 0, 1, 2, 3, and $S_0 = S_1 = S_2 = S_3$ prior to each flash.

The paired-flash oxygen evolution experiments of Joliot et al. [5], Kok et al. [4], Bouges-Bocquet [6] and Diner [7] have measured the overall relaxation time for Photosystem II. The results of these experiments, coupled with the fluorescence data of Zankel [8] and of Joliot et al. [9], show that the rate limiting step for the recovery of photoactivity in Photosystem II units is a function of the oxidation state of the S enzyme. Photosystem II units in states S_0, S_1, or S_2 prior to a flash regain photoactivity as Q^- is reoxidized, indicating that reactions occurring on the reducing side of Photosystem II are rate-limiting. However, for Photosystem II units in S_3 prior to a flash, the rate limitation shifts to the oxidizing side of the reaction center. These results suggest that the time course for the rereduction of Z^+ may vary with the oxidation state of S.

In the experiments reported in this communication we have used dark-adapted chloroplasts to measure the time course for the reaction $Z^+ + S_n \xrightarrow{k_n} Z + S_{n+1}$ for each of the first 4 flashes. Our results are compatible with the oxygen evolution and fluorescence data described above and suggest that the rate (k_n) of hole transfer from Z^+ to the S manifold decreased as the number of positive charges accumulated in the S enzyme increases.

2. Materials and methods

Spinach chloroplasts were prepared as described previously [2]. Ferredoxin (50 μg/ml) and NADP (10^{-3} M), obtained from Sigma, were included as the acceptor system. The temperature in all experiments was 22°C.

Signal averaged e.p.r. measurements were made with the modified Varian E-3 instrument described previously [2]. The instrument response was limited at 100 μsec. Saturating flashes (10 μsec at half height) were transmitted to the e.p.r. cavity via the 8-foot lucite light pipe as previously described [2]. Signal II amplitude was monitored at the low field peak of Signal II (3379 G in these experiments).

Chloroplasts were dark-adapted by incubation for at least 10 min in absolute darkness at 0°C. The reservoir of dark-adapted chloroplasts was connected

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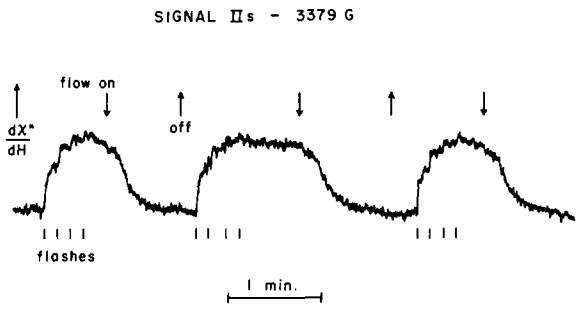

Fig.1. A control experiment designed to test the effectiveness of the flow system in maintaining dark adaptation conditions in the chloroplast reservoir. Single, 10 μsec flashes indicated by vertical lines; new samples flowed into the e.p.r. cavity as indicated by arrows. Instrumental conditions: microwave power, 25 mW; modulation amplitude, 4G; time constant, 1.0 sec.

via tygon tubing wrapped in black tape to a Scanlon (Costa Mesa, Ca.) flow e.p.r. flat cell (S-814) positioned in the e.p.r. cavity. Following a flash sequence, a fresh dark-adapted sample was flowed into the cell, the flash sequence was repeated, and the results were accumulated in the signal averager.

The trace shown in fig.1 is the result of an experiment designed to test the ability of the flow system to maintain rigorous dark conditions. Chloroplasts prepared with low dark levels of Signal IIs [10] are transferred to the flow system. Single flashes are applied as indicated to saturate the formation of Signall IIs is the first sample and then a second sample is transferred to the flat cell from the reservoir. The signal II magnitude returns to the level observed prior to illumination of the first sample, indicating that we are able to maintain dark adaptation in the flow system. A third cycle is also shown in fig.1 to demonstrate reproducibility.

3. Results and discussion

Fig.2(a) and (b) show the effect of a group of four flashes (spaced 10 msec apart) on Signal IIvf amplitude and decay kinetics in two different preparations of dark-adapted chloroplasts. The Signal IIvf amplitude owing to the first flash is low (< 40%) compared to subsequent flashes; the poor signal/noise ratio precludes extraction of a decay time. The slight non-decaying Signal II component observed on the first flash may correspond to Signal IIf formation in Photosystem II units whose oxygen evolving centers were damaged during chloroplast preparation [1]. The amplitude of the Signal IIvf transient due to the second flash is 80–90% of that observed on the third flash. However, the decay halftimes for these two transients differ significantly: 400 μsec for the second flash and 1 msec for the third flash. The Signal IIvf amplitude due to the fourth flash is reduced by about 40–45% compared to that of the third flash; however the decay times are similar. As shown in fig.2(c), a repeat of the experiments of fig.2(a) and (b) in the presence of 1×10^{-5} M DCMU* eliminates all flash induced changes in Signal II except for a rapid transient on the first barely observable above the noise, and the baseline shift corresponding to Signal IIvf formation.

The limitations imposed by the 100 μsec instrument constant make an unambiguous interpretation of the results of fig.2 difficult. However the following conclusions seem clear and are consistent with recent

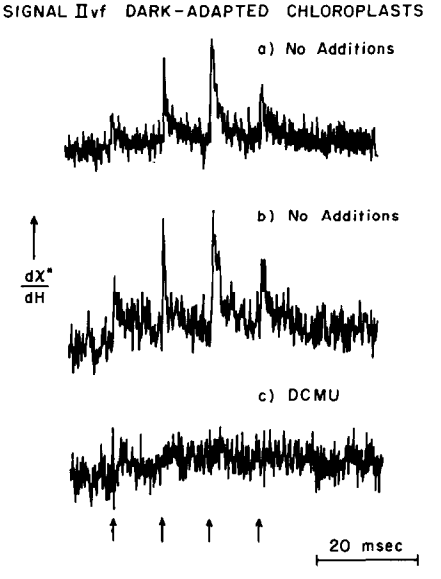

Fig.2. Signal IIvf formation in dark-adapted spinach chloroplasts; saturating, 10 μsec flashes given as indicated by upward arrows. (a) and (b) no additions; (c) + 10^{-4} M DCMU. Instrumental conditions: microwave power, 100 mW; modulation amplitude, 4 G; time constant, 100 μsec. Each trace is the average of 1024 events.

* *Abbreviations:* DCMU, 3-(3,4-dichlorophenyl)-1, 1-dimethylurea.

oxygen evolution, absorption, fluorescence and luminescence data. On each flash, regardless of the oxidation state of the S enzyme, the formation of Signal IIvf (oxidation of Z) is fast compared with 100 μsec. Glaser et al. [11] from absorption measurements in the red region of the spectrum, and van Gorkom and Donze [12], arguing from the luminescence data of Zankel [13], have estimated that P680 is rereduced with a halftime of 35 μsec. Duysens and co-workers [14] have suggested much shorter times (1–5 μsec) on the basis of fast fluorescence measurements. Either time is consistent with our results. However, the results of fig.2 argue against a major component of $P680^+$ being rereduced in 120-200 μsec as recently suggested by Döring [15].

In dark adapted chloroplasts S_3 is formed in the major fraction of traps on the second flash and S_4 on the third [4]; therefore we attribute the 400 μsec decay of Signal IIvf observed on the second flash to S_3 formation and the 1 msec time on the third flash to the formation of S_4. The 1 msec time we observe for the reaction $Z^+ + S_3 \rightarrow Z + S_4$ is similar to the 0.9 msec time measured polarographically by Joliot, Hofnung and Chabaud [16] for the Photosystem II mediated release of oxygen and also to the 1.2 msec time measured by Bouges-Bocquet [6] for the relaxation reaction $S_3 \rightarrow S_4 \rightarrow S_0$ in paired-flash oxygen evolution experiments. In the light of these data our results indicate that the oxidation of water occurs rapidly upon the appearance of S_4; i.e., water oxidation is rate-limited by the 1 msec time constant observed for the formation of S_4. Sinclair and Arnason [17] have also concluded that the rate-limiting step in photosynthetic water oxidation does not involve breaking of water O–H bond, on the basis of deuterated water experiments. Therefore our data are consistent with a model in which Photosystem II regains photoactivity following the third flash on dark-adapted chloroplasts as the reaction $Z^+ + S_3 \rightarrow Z + S_4$ occurs.

As noted above, we attribute the 400 μsec decay for Signal IIvf observed following the second flash to the formation of S_3. This time is significantly faster than S_4 formation but comparable to the 400–500 μsec time measured by Kok et al. [4] for the relaxation of Photosystem II following the second flash. Zankel [8] has also detected 400–500 μsec decay kinetics for fluorescence under the same conditions. These results, taken together, indicate that in dark-adapted chloroplasts the rereduction of Z^+ and the reoxidation of Q^- proceed at approximately the same rate following the second flash.

The results discussed above suggest that the oxidation rate of the S enzyme by Z^+ is dependent on the number of charges stored in S: the higher the oxidation state of S, the slower the reduction of Z^+. This 'capacitor' effect has been considered in detail for photosynthetic system by Tributsch [18] and by van Gorkom and Donze [12] and may account for the lower amplitude for Signal IIvf that we observe for the first and fourth flashes. The first flash produces 75% S_2 and 25% S_1; both states are low oxidation states of S and may be formed in a time comparable to or less than our 100 μsec time constant. The fourth flash produces roughly 60% S_1 and 40% S_4; the formation of S_4 would correspond to the fairly long decay for Signal IIvf observed after the fourth flash but rapid S_1 formation (within the instrument time constant) would diminish the signal amplitude. The fluorescence results of Zankel [8] strengthen this interpretation. He observed that, although the reset time for oxygen evolution after the first flash (200–250 μsec) is roughly half that following the second flash, this recovery still appears to correlate with acceptor side reactions. Similarly Diner [7] has shown that, under conditions where the normal acceptor-side rate limitation is relieved, the reset time for the oxygen system in low S oxidation states may approach 100 μsec.

Based on the above discussion the following set of equations summarizes the model that we propose for reactions occurring on the water side of Photosystem II in dark-adapted chloroplasts:

(1) $P680^+ + Z \rightarrow P680 + Z^+$	$t_{1/2} \ll 100$ μsec
(2) $Z^+ + S_0 \rightarrow Z + S_1$	$t_{1/2} \lesssim 100$ μsec
(3) $Z^+ + S_1 \rightarrow Z + S_2$	$t_{1/2} \lesssim 100$ μsec
(4) $Z^+ + S_2 \rightarrow Z + S_3$	$t_{1/2} \approx 400$ μsec
(5) $Z^+ + S_3 \rightarrow Z + S_4$	$t_{1/2} \approx 1$ msec
(9) $S_4 + 2H_2O \rightarrow S_0 + 4H^+ + 4_e + O_2$	$t_{1/2} < 1$ msec

It is important to point out, however, that under steady state conditions other reactions, for example proton flux or the membrane electric field, may exert influence on these reactions and alter time courses.

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