Kinetics of the oxidation–reduction reactions of the photosystem II quinone acceptor complex, and the pathway for deactivation

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When the photosystem II quinone acceptor complex has been singly reduced to the state $Q_AQ_B^-$, there is a 22 s half-time back-reaction of Q_B^- with an oxidized photosystem II donor (S_2), directly measured here for the first time. From the back-reaction kinetics with and without inhibitors, kinetic and equilibrium parameters have been estimated. We suggest that the state $Q_AQ_B^-$ of the complex is formed by a second-order reaction of vacant reaction centers in the state Q_A^- with plastoquinone from the pool, and discuss the physico-chemical parameters involved.

Photosynthesis *Fluorescence yield* *Kinetics* *Plastoquinone* *Two-electron gate* *Semiquinone*

1. INTRODUCTION

Electron transport on the acceptor side of PS II is thought to proceed through a two-electron gating mechanism [1,2]. This mechanism involves a primary quinone acceptor (Q_A), which can react only by one-electron processes. The redox state of Q_A is the primary modulator of fluorescence from PS II [3]. A secondary quinone acceptor (Q_B) also functions in the gating mechanism by allowing one electron to be stabilized within the Q_A/Q_B pair until a second electron arrives from PS II. When the second electron arrives, a fully reduced plastoquinol can be formed and is released to the pool. Velthuys [4,5] has suggested that the gating mechanism should be thought of as involving a binding site (the Q_B site) for plastoquinone from the pool; plastoquinone and plastoquinol would be in exchange with this site, while plastosemiquinone would be stabilized by firm binding to the site. Inhibitors such as DCMU and *o*-phenathroline were proposed to inhibit PS II by competing with plastoquinone and plastoquinol for the Q_B site.

A formal description of the mechanism of the two-electron gate in terms of this hypothesis requires measurement of the kinetic and equilibrium binding constants for the reactions of plastoquinone and plastoquinol (and also of inhibitors) at the Q_B site, and also the kinetic and equilibrium constants for the reaction of Q_A^- with Q_B when one or two electrons have been accumulated from PS II. Here, we have investigated the reactions of the quinone acceptor complex when the system has stored one electron and formed Q_B^- in most centers. The stored electron does not disproportionate with other centers, or with the pool at a significant rate. The electron in the acceptor complex can back-react with an oxidizing equivalent (S_2) stored on the donor side of PS II. This observation shows that deactivation of the donor complex of PS II as detected by O_2 polarographs [6–8] involves electron transfer by back-reaction from Q_B^- on the acceptor side. Our results support and extend studies [9,10], where thermoluminescent

Abbreviations: PS II, photosystem II; Q_A, the primary quinone acceptor of PS II; Q_B, the secondary quinone acceptor of PS II; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; chl, chlorophyll

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glow curves and kinetic components of delayed fluorescence [11] were correlated with a back-reaction from Q_B^- to the donor side of PS II. The results are discussed in terms of a second-order mechanism for reduction of plastoquinone at the Q_B site, and preliminary values for the physico-chemical constants are estimated.

2. MATERIALS AND METHODS

Leaves from incubator grown pea seedlings (21 days at 18°C) were ground for 10 s in 0.4 M sucrose, 20 mM Hepes (pH 7.6), 15 mM NaCl, 5 mM $MgCl_2$, 2 mM EDTA, 2 mg bovine serum albumin/ml. The suspension was filtered through cheese cloth, centrifuged for 10 min at 2000 × g, and resuspended in 0.4 M sucrose, 20 mM Hepes (pH 7.6), 15 mM NaCl (SHN). The suspension was diluted in 0.1 M sucrose, 20 mM Hepes (pH 7.6), 20 mM KCl, 5 mM $MgCl_2$, 50 μM ferricyanide, to a final concentration of 0.15 mg chl/ml. This was then stirred slowly on ice in absolute darkness for 1 h, centrifuged for 10 min at 2000 × g, and resuspended in SHN. Chloroplasts from this procedure were found to have < 5% of their centers as Q_B^-, as indicated by the extent of DCMU-induceable Q_A^- formation [2]. The concentration of ferricyanide used in the incubation was selected to give a minimum amount of Q_B^-, while at the same time avoiding any detectable formation of the high-potential acceptor discussed in [12].

The fresh chloroplast suspension was diluted in the dark to 5 μg chl/ml in a dark reaction vessel at 22°C containing 100 ml of 0.1 M sucrose, 20 mM Hepes (pH 7.6), 10 mM NaCl, 5 mM $MgCl_2$, and 100 μM methylviologen. A flow cuvette (illumination volume 0.6 ml) was filled from this vat by gas pressure on top of the liquid in the vat. Reversing the pressure emptied the cuvette into the vat at the end of each measurement. The gas pressure was varied under computer control by switching a set of solenoid valves.

The level of fluorescence of the sample in the cuvette was measured at 685 nm (10 nm bandwidth), using a weak measuring flash (~1% of the centers sampled) [13]. This measuring flash could be given at variable times after a series of saturating flashes. Both the measuring flash (Stroboslave, General Radio) and the saturating flash (FX-201, EG + G) were blocked by Corning CS 4-96 filters, and were of 2.5 μs duration at half-maximal peak height.

3. RESULTS AND DISCUSSION

The high fluorescence yield of PS II, associated with reduction of Q_A on illumination, decreases with half-times of 100 or 200 μs after one or two actinic flashes, respectively (fig.1). These kinetics reflect primarily the oxidation of Q_A^- by the secondary quinone acceptor. After the first flash Q_A^- is oxidized by Q_B, and after the second flash by Q_B^- [14]. In fig.2A, the fluorescence yield was measured at 150 μs after a series of actinic flashes. The lower values after odd number flashes reflect the faster oxidation of Q_A^- by Q_B relative to oxidation of Q_A^- by Q_B^- after even numbered flashes. Under these experimental conditions, < 5% of the centers were in the state Q_B^- in the dark prior to the actinic flashes (see section 2). In fig.3, one actinic flash was given so that nearly all the centers would be in the state Q_B^-. The sample was then held in the dark for the number of seconds specified at the right of the figure, and then given a sequence of flashes as in fig.2A. At short dark times, the fluorescence as a function of flash number showed oscillations, but these were reversed in phase compared with the dark state, indicating the presence of Q_B^- in most of the centers prior to the assay

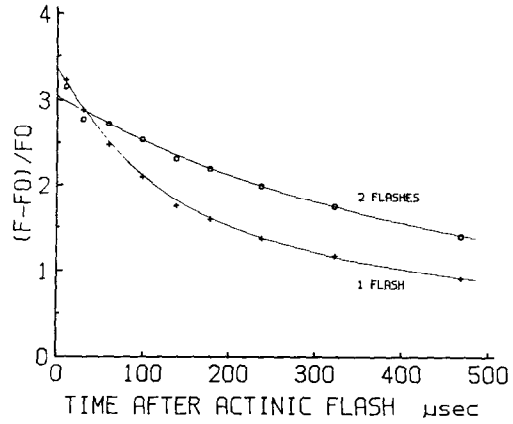

Fig.1. Decay of the high fluorescence state after 1 or 2 saturating flashes. A dark time 250 ms was allowed between the 2 saturating flashes. Data points at times < 60 μs were corrected for the tail of the saturating flash during the measuring integration period. Other experimental conditions as in section 2.

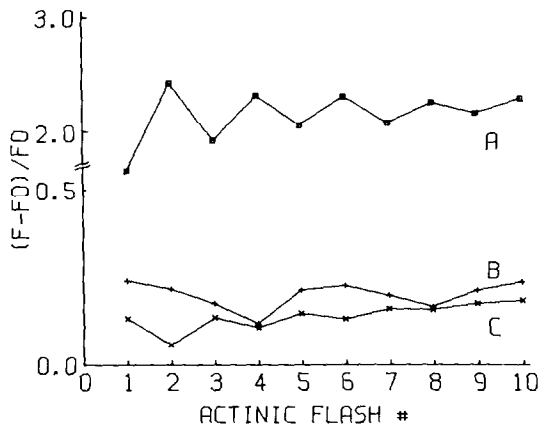

Fig.2. Oscillations in the level of fluorescence measured after each of a series of saturating flashes: (A) fluorescence measured at 150 μs, after each of a series of saturating flashes given at 4 Hz; (B) fluorescence measured at 1 s after each of a series of saturating flashes given at 1 Hz; (C) same as (B) except that 10 mM NH_2OH was added 1 min prior to the start of the sequence. Curves (B) and (C) are each the average of 4 expts. Note that the scale for (A) is different from that for (B) and (C). Both scales are linear.

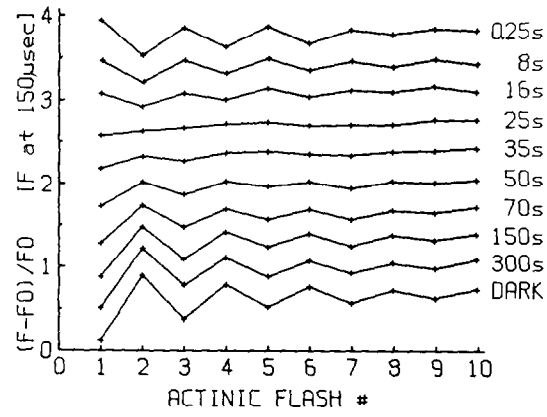

Fig.3. Oscillations of the level of fluorescence, after each of a series of saturating flashes, as a function of the time in the dark after 1 saturating flash. One saturating flash was given, followed by the dark interval indicated at the right, followed by the assay sequence as in fig.2A. For all of the sequences, the mean values of the points was ~2 (F-FO)/FO; the curves have been offset for presentation.

(fig.3). As the dark incubation time was increased, the amplitude of the oscillation decreased until at 25 s there appeared to be no oscillations. We interpret this as showing that ~50% of the centers had lost Q_B^- after 25 s. At longer dark times, the phase of the oscillations seen after dark adaptation reappears, and the amplitude approaches that of the dark-adapted sample. The kinetics of loss of Q_B^- can be measured by plotting the change in phase of the oscillation as a function of time (fig.4A). Here the scale 'oscillation' represents a linear proportion of the centers which have had their Q_B^- oxidized. This scale is linear because only small differences in the fluorescence signal were measured about a common mean, so that the non-linearity of the relation between the fluorescence signal and Q_A^- caused by connectivity of the PS II center pigments, seen when measuring over large ranges [15–17] was minimized. The insert to fig.4 shows that the oxidation of Q_B^- is first-order, with a half-time of 22 s for ~80% of the centers. This rate of oxidation of Q_B^- is dramatically slowed, to give a half-time of > 10 min after addition of 30 μM NH_2OH (fig.4B), indicating that the first-order decay of 22 s half-time in the absence of NH_2OH represents a back-reaction from Q_B^- to an oxidant on the donor side of PS II (S_2). Q_A^- is known to back-react with S_2 [18], and in the presence of DCMU (fig.4C), Q_A^- is oxidized with a half-time of 1.5 s, as judged from the decay of the high fluorescence state. This measurement does, however, suffer from the problem of non-linearity discussed above, and for this reason, the half-time of 1.5 s should be considered a lower limit. The ratio of the back-reaction half-times of Q_B^- and Q_A^- is 15. If the back-reaction from Q_B^- is through Q_A^-, and the state of Q_A^- is not modified by DCMU, then the ratio of the two half-times is equal to the equilibrium constant for the sharing of the one electron between Q_A and Q_B. For a simple model of the two-electron gate, with Q_B as a bound species, this gives a value for $K_{1,app} = 15$ (see below, eq. (2)), and for $E_m(Q_B/Q_B^- - Q_A/Q_A^-) = 70$ mV. If we take the E_m at pH 7.6 for the couple Q_A/Q_A^- to be -30 mV [19,20], then the E_m at pH 7.6 for the couple Q_B/Q_B^- would be ~40 mV. This estimation assumes a large value for the association constant (K_o) for binding of plastoquinone to the Q_B site (see below).

If the equilibrium constant between Q_A and Q_B

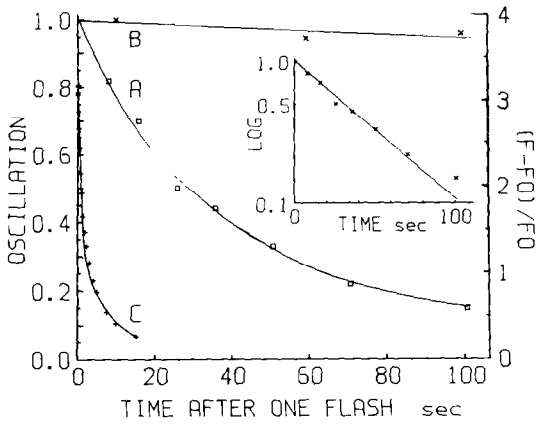

Fig.4. Decay of the oscillations of fig.3, or of the variable fluorescence, as a function of dark time between one saturating flash and the assay. (A) The data are from fig.3. The extent of oscillation was measured from the sum of the differences of the measured fluorescence in each sequence, compared to a range established by the difference between similar sums for control sequences (0.25 s and DARK). (B) The same as (A) except that 30 μM NH_2OH was present. (C) Fluorescence measured after one saturating flash in the presence of 10 μM DCMU (right scale). The insert shows the points of curve (A) plotted on a semilogarithmic scale.

for one electron is 15, then when equilibrium is reached (certainly in < 1 s), the fluorescence measured after each of a series of actinic flashes should reflect the concentration of Q_A^- in the equilibrium mixture. An experiment to test this prediction is shown in fig.2 curves (B) and (C). The points of curve (B) show a period of 4 oscillations, correlated with the number of centers in states S_2 and S_3 at long times after flash illumination [7,21]. The decay of the residual fluorescence component after one flash had a half-time of 22 s (not shown), identical to the kinetic of Q_B^- above. One-half of this residual fluorescence component was directly attributable to Q_A^- (in equilibrium with Q_B) as shown by the experiment below, while the other half may still have been due to S_2 [7,17]. Fig.2C shows that when 10 mM NH_2OH was added (this would eliminate any modulation of the fluorescence signal by the donor side of PS II), the period of 4 oscillations disappeared and a binary oscillation was evident (see also [17]). The fluorescence signal was higher after odd actinic flashes due to the equilibration of the one electron shared between Q_A and Q_B. It was lower after even actinic flashes due to a large equilibrium constant favoring reduction of Q_B^- for the two electrons shared between Q_A and Q_B [8,22]. The difference between the fluorescence signal after the first and second actinic flashes is 2.5% of the full scale change. After correcting for the non-linearity effect (calculated by comparing the increase in the area above the fluorescence induction curve and the level of the fluorescence signal [23]), this corresponds to 5% of the centers in the state Q_A^-. Since Q_A is reduced 5% of the time, the equilibrium constant between Q_A and Q_B for the sharing of one electron must be ~20, a value close to the value of 15 calculated from the back-reaction rates.

A half-time of 22 s for the back-reaction from Q_B^- to S_2 is in approximate agreement with reported measurements of half-times for the resetting of S_2 to S_1, as measured with O_2 polarography [6–8]. On the basis of such measurements, a value for the equilibrium constant above ~20 was calculated [8,24] on the assumption that the electron donor to S_2 was Q_B^-. Our measurements show that this assumption was justified, and for the first time demonstrate unambiguously that the source of electrons for deactivation of S_2 is Q_B^-. In view of this demonstration, the value of $K_{1,app} = 15$–20 we have determined can be used with greater confidence in calculations related to the redox chemistry of the components of the acceptor complex.

Recent studies on inhibitor binding to the Q_B site in competition with plastoquinone, have suggested that K_1 must be > 100 (in preparation). Since this value for K_1 is larger than that for $K_{1,app}$ measured above, the value of K_o must be relatively small, so that most centers without Q_B^- have a vacant binding site.

$$Q_A^- + \text{plastoquinone} \overset{K_o}{\rightleftharpoons} Q_A^- Q_B \overset{K_1}{\rightleftharpoons} Q_A Q_B^- \quad (1)$$

$$K_{1,app} = [Q_A Q_B^-]/([Q_A^-] + [Q_A^- Q_B]) \quad (2)$$

$$K_1 = [Q_A Q_B^-]/[Q_A^- Q_B] = [Q_A Q_B^-]/([Q_A^-] \cdot [\text{plastoquinone}] \cdot K_o) \quad (3)$$

An approximate value for K_o can be calculated using eq. (2) and (3), if the concentration of the pool plastoquinone and K_1 and $K_{1,app}$ are known (for a discussion of these binding constants see [25] and Stein, R.R. and Wraight, C.A. [manuscript in preparation]). In our preparations there are 8 plastoquinone molecules/PS II reaction center as determined by comparison of the area over the fluorescence transients from chloroplasts inhibited with DCMU to the area in chloroplasts inhibited with Hg^{2+} and CN^- [26]. If we assume that there are 400 chl molecules/PS II reaction center, and that the ratio by weight of the non-chlorophyll lipids to chlorophyll is 3.2 [27], then [plastoquinone] in the lipid phase of the membrane is 5 mM and [PS II] reaction centers is 0.7 mM. Substituting $K_{1,app} = 15$, $K_1 > 100$ and [plastoquinone] = 5 mM, into eq. (2) and (3), yields $K_o < 35\ M^{-1}$.

If K_o is as small as our experiments suggest, then the number of Q_B sites occupied by plastoquinone in the dark state is < 15%. This means that after the first flash (fig.1) there must be second-order reaction between PS II reaction centers and plastoquinone from the pool. The rate constant for this reaction ($10^6\ M^{-1}.s^{-1}$, calculated from fig.1, and from the concentrations estimated above) is close to that expected for a diffusion limited reaction, assuming appropriate values for the radii (1–4 nm) and diffusion constants of the reacting molecules (10^{-9}–$10^{-10}\ cm^2.s^{-1}$, see [28]). A value for K_1 of > 100 also means that the redox midpoint potential for the couple Q_B/Q_B^- is at least 120 mV more positive than that for the couple Q_A/Q_A^-. Clearly the actual value of $E_m(Q_B/Q_B^-)$ will depend on the value chosen for $E_m(Q_A^-)$. We have used a value for E_m (pH 7.6) of −30 mV above, from [19,20,29], rather than the value of −300 mV suggested in [30], since the latter value was obtained in the absence of mediators. Using this value, $E_m(Q_B/Q_B^-)$ is > 90 mV.

If the association constant (K_r) for binding of plastoquinol to the Q_B site has a value similar to the association constant for plastoquinone (K_o), and given that for the pool, E_m(plastoquinone/plastoquinol) = 84 mV [31] at pH 7.6, then $E_m(Q_B^-/Q_B^{2-})$ is < 78 mV. From these values, the semiquinone stability constant for Q_B^- in equilibrium with bound quinone and quinol at the catalytic (Q_B) site would be $K_s(Q_B^-) > 1.6$, compared with a value for $K_s \simeq 10^{-10}$ estimated [32] for the stability constant of semiquinone free in the membrane. However, this does not necessarily mean that Q_B^- will be formed stably in all centers under equilibrium conditions. Assuming that $K_o = K_r = 35\ M^{-1}$, we would expect a maximal amount of Q_B^- when the plastoquinone pool is one-half reduced, and this maximum would correspond to 12% of the centers with Q_B^-. If we had assumed a more negative value for $E_m(Q_A/Q_A^-)$ (see above), both the calculated stability constant and the maximal amount of Q_B^- expected would have been less. We are now undertaking equilibrium experiments to determine the extent of Q_B^- formation in the dark at various redox potentials.

In the discussion above we have ignored the role of protolytic reactions. The apparent equilibrium constants were derived from kinetic processes with half-times > 1 s, and probably reflect activities of reactants which had reached protonic equilibrium. There is some ambiguity about the role of protons in the acceptor side reactions, and until this is resolved, a more detailed consideration would seem premature.

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