Biochimica et Biophysica Acta, 590 (1980) 373–384
© Elsevier/North-Holland Biomedical Press

BBA 47837

BINARY OSCILLATIONS IN THE RATE OF REOXIDATION OF THE PRIMARY ACCEPTOR OF PHOTOSYSTEM II

JANE M. BOWES and ANTONY R. CROFTS

Department of Physiology and Biophysics, 524 Burrill Hall, University of Illinois, Urbana, IL 61801 (U.S.A.)

(Received August 22nd, 1979)

Key words: Photosystem II acceptor; Reoxidation; Fluorescence; Primary acceptor; Binary oscillation; (Spinach chloroplast)

Summary

The decay of fluorescence yield following each of a series of saturating laser flashes has been used to monitor the kinetics of reoxidation of the primary acceptor of Photosystem II under conditions of varied redox potential.

1. In dark-adapted chloroplasts, a damped binary oscillation as a function of flash number was observed in the kinetics of the decay of the fluorescence yield. The decay was faster on odd than on even-numbered flashes.

2. In the presence of low concentrations of 1,4-benzoquinone, the oscillation was more marked, and over the range approx 200–350 mV, independent of redox potential. The decay following flash 1 under these conditions had a half-time of approx. 200–400 μs. The decay following flash 2 was decelerated; the initial rate was up to 10-fold slower than after flash 1.

3. We suggest that the kinetics following a single flash reflect the rate of the reaction $Q^-B \rightarrow QB^-$, and following the second flash, $Q^-B^- \rightarrow QB^{2-}$. Benzoquinone at low concentrations oxidises a residual fraction of B^- which is usually reduced in the dark before the flash sequence.

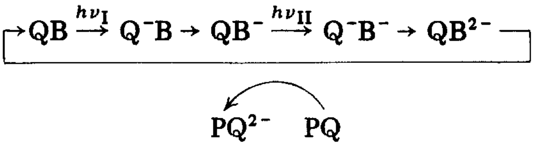
4. A faster component in the decay ($t_{1/2} \approx 140$ μs) following the first flash titrated in over the range $E_h > 350$ mV. The binary oscillation was still apparent but delayed by one flash.

5. We discuss the relative redox potentials of the couples B/B^- and B^-/B^{2-}, and the role of the component which titrates in at $E_h > 350$ mV.

Abbreviations: PS II, Photosystem II; Tricine, *N*-tris(hydroxymethyl)-methylglycine.

Introduction

It is now generally accepted that electrons are transferred via a two-electron gate, B [1—3], from the primary acceptor of PS II, Q, to the plastoquinone pool according to the following scheme, in which the involvement of H^+ is ignored:



$$\rightarrow QB \xrightarrow{h\nu_I} Q^-B \rightarrow QB^- \xrightarrow{h\nu_{II}} Q^-B^- \rightarrow QB^{2-} \quad PQ^{2-} \quad PQ$$

Mathis and Haveman [4] and Pulles et al. [3] identified B as a specialised plastoquinone molecule by characteristic absorption changes in the ultraviolet region of the spectrum. In a sequence of flashes, B was reduced to a semiquinone on uneven flashes ($h\nu_I$, above). A biphasic oscillation as a function of flash number in absorption at 310 nm was observed in chloroplasts treated with hydroxylamine to inactivate the donor side of the photosystem [3,5], and in the amplitude of a fast phase of the decay of the absorption change in chloroplasts treated with alkaline Tris buffer and supplied with artificial donors [4,6]. Both these observations were consistent with the formation of B^- on uneven flashes. In untreated chloroplasts, absorption changes in this region are complicated by a contribution from the S states [3,7], and although the decay of the absorption change following the first two flashes was the same as in chloroplasts washed with Tris buffer and therefore compatible with the above model, on subsequent flashes the magnitude of the fast phase was anomalously large [4].

The kinetics of reoxidation of Q^- can also be followed by monitoring the fluorescence yield at varying times following a saturating flash [8—11]. We have used this technique, and observe a marked biphasic oscillation as a function of flash number in the kinetics of oxidation of Q^- in untreated dark-adapted chloroplasts. We discuss our results in terms of a difference in the electron transfer rate from Q^- depending on the redox state of the secondary acceptor B.

Methods

Preparation of chloroplasts. Chloroplasts were prepared from spinach (*Spinacae oleracea*) as previously described [12], and suspended in a medium containing 0.4 M sucrose, 10 mM NaCl plus 50 mM phosphate buffer (pH 7.8) for measurements of fluorescence. Chloroplasts were also prepared from redroot pigweeds (*Amaranthus retroflexus*) as described by Arntzen et al. [13], and suspended in 0.1 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$ plus 10 mM Tricine/NaOH, pH 7.8. All measurements were performed at room temperature on chloroplasts which had been dark adapted for 10 min.

Fluorescence yield. Measurements of fluorescence yield following a sequence of saturating laser flashes were performed according to a method described by Joliot [14]. The fluorescence was monitored at varying times (50 μs—10 ms) following a saturating flash by a non-actinic measuring flash (General Radio Stroboslave 1539A), screened by a blue filter (Corning 9782), and detected at right angles to the actinic flash (see below), by a gated photomultiplier (EMI 9558QB), screened by a red cut-off filter (Schott RG665) and an interference filter with an emission peak at 695 nm (Balzer's B-40). Excitation of photosynthesis was provided by a Q-switched laser (Laser Associates, Slough, U.K.; model 252 YAG laser, frequency doubled to emit a 530 nm, pulse width at half-height 20 ns). The output from the photomultiplier was stored in a microprocessor-controlled data samples (designed and built by Mr. G.V. Tierney), and stored values could be printed as a set of three digit decimal numbers on a teletype.

The data sampler consisted of a fast-responding input amplifier, sample and hold, 8-bit analog to digital converter, 256 byte memory, LED numerical display and serial output, all under the control of an M6800 microprocessor running under a firmware program. Appropriate timing circuits ensured that the peak amplitude of the flash was sampled.

The value of F_0 for each set of kinetic points was measured before actinic excitation by monitoring the fluorescence induced by the non-actinic measuring flash using dark-adapted chloroplasts.

Redox potential. Redox potentials of the reaction media were adjusted with $K_3Fe(CN)_6/K_4Fe(CN)_6$ mixtures and monitored by a conventional platinum/calomel electrode pair using a pH meter (Pye model 57, Cambridge, U.K.). The electrodes were routinely checked using an equimolar mixture of ferri- and ferrocyanide at 10 mM concentration in 100 mM KCl. The potential for this couple was taken as 436 mV. Ascorbate was used as reductant in some experiments for potentials at the lower end of the buffering range for the ferri-/ferrocyanide couple.

Results and Discussion

Decay of fluorescence following one or two laser flashes

Fig. 1 shows the fluorescence yield of dark-adapted spinach or pigweed chloroplasts illuminated by one or two saturating laser flashes, measured at times between 50 μs and 10 ms after the flash. Over this time scale, the decay of fluorescence largely reflects the rate of reoxidation of the primary acceptor Q, which quenches fluorescence in its oxidised form only [8]. The kinetics of the decay following a single flash, or on repeated flashing, are similar to those previously reported [9,10]. The decay observed after a second flash was more complex and markedly slower than the decay after one flash. This effect was also observed by Zankel [10], but was attributed to different quenching effects of the S states (see below).

Fig. 1 also shows that in the presence of low concentrations of 1,4-benzoquinone, shown by Wollman [15] to oxidise the secondary acceptor B, the rate of decay of fluorescence following the first flash was considerably more rapid. This effect was somewhat variable from preparation to preparation. It appeared to be related to some extent to the degree of intactness of the chloroplasts, and to the concentration of benzoquinone. This result is compatible with the data of Mathis and Haveman [4] and suggests that a fraction of B, reduced in

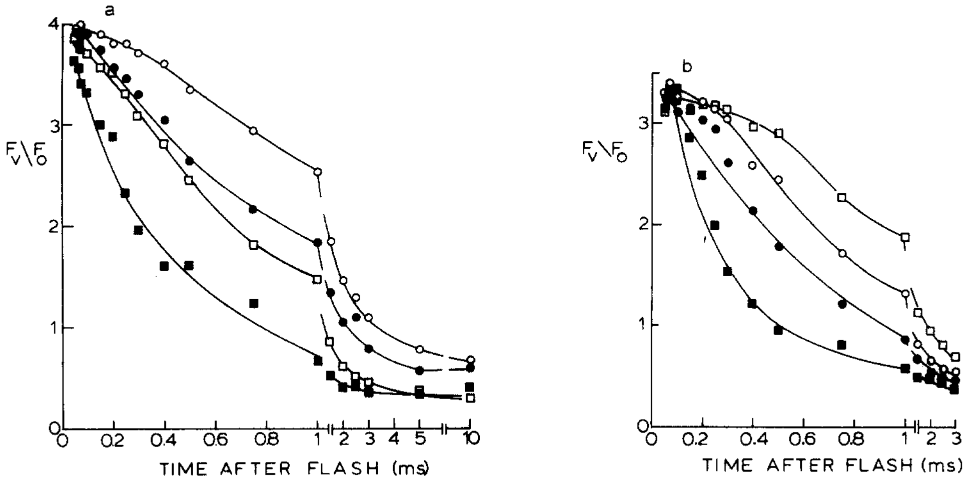


Fig. 1. Decay of fluorescence following one or two saturating laser flashes given to dark-adapted chloroplasts. Spinach (a) or pigweed chloroplasts (b) were preincubated in the dark for 10 min in the absence (○,●), or presence (□,■) of 200 μM benzoquinone (BQ). Fluorescence was detected between 50 μs and 10 ms (3 ms in experiments shown in (b)) following one (●,■) or two (○,□) laser flashes (frequency : 1/s). Spinach chloroplasts were suspended in 0.4 M sucrose, 10 mM phosphate buffer (pH 7.8) plus 10 mM NaCl. Chlorophyll concentration 6.1 μg · ml^{-1}. Pigweed chloroplasts were suspended in 0.1 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$ plus 10 mM Tricine/NaOH, pH 7.8. Chlorophyll concentration 5.0 μg · ml^{-1}.

the dark, was reoxidized by benzoquinone [1—4,15]. In fig. 2 the effect of the kinetics of the first two flashes of varying the concentration of benzoquinone at fixed ambient redox potential is shown. Even at very low concentrations (2 μM), preincubation with benzoquinone increased the rate of decay after the first flash (not shown). At 20 μM the effects of benzoquinone on the first flash were more pronounced and the kinetics of decay after the second flash were slowed when compared to control chloroplasts; at 200 μM, a faster decay after the second flash became apparent (as in Fig. 1). We interpret these results as showing that benzoquinone can mediate the oxidation in the dark of B^- even at low concentrations (cf., Ref. 15), and that at higher concentrations (200 μM or greater, depending on the preparation), the rate is sufficiently rapid to oxidise a substantial proportion of B^- in the time between flashes.

It is also noticeable in Figs. 1 and 2 that the markedly biphasic nature of the decay after a single flash in control chloroplasts, or of the decay after multiple flashes (not shown), largely disappeared after preincubation with benzoquinone. This strongly suggests that the biphasic decay is a reflection of the inhomogeneity of the B pool. The variability from preparation to preparation of (a) the extent of oscillation in untreated chloroplasts; (b) the contribution of the slow component to the decay after the first flash in these chloroplasts, and (c) the stimulation of the decay after the first flash by preincubation with benzoquinone, were consistent with the idea that the variability reflected the proportion of centers associated with B^-. As this proportion approached 50%, the differences between the decay kinetics after the first and second flash disappeared. It would seem likely therefore that in benzoquinone-

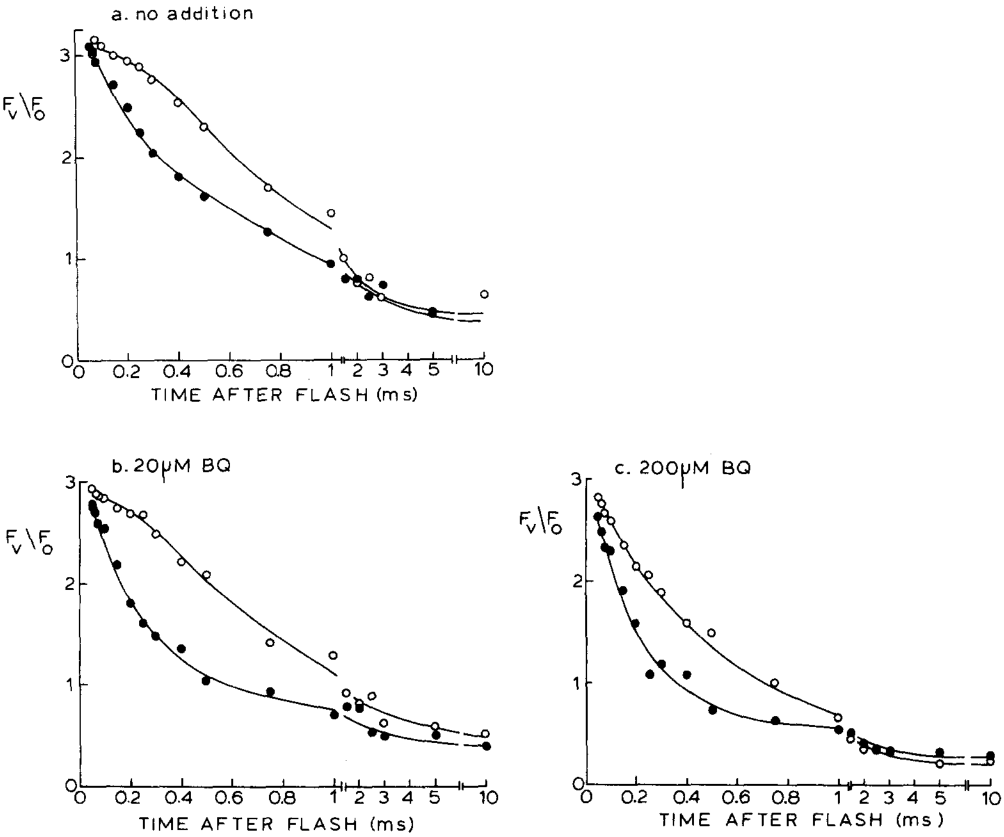


Fig. 2. Effect of concentration of benzoquinone on the decay of fluorescence following one or two saturating laser flashes given to dark-adapted chloroplasts. Protocol as described in legend to Fig. 1. Spinach chloroplasts were dark adapted with (a) no additions; (b) 20 μM, or (c) 200 μM benzoquinone (BQ). For (b) and (c) the redox potential was adjusted to 291 mV with $K_3Fe(CN)_6$. Chlorophyll concentration 7.5 μg · ml^{-1}.

treated chloroplasts, the decay of fluorescence following a single flash is determined largely by the rate of the reaction $Q^-B \rightarrow QB^-$, or following two flashes, by the reaction $Q^-B^- \rightarrow QB^{2-}$. The latter reaction appears to be considerably slower than the former. If we assume that the proportion of the B system in the fully oxidised state is maximal when the difference kinetics between the decays after one and two flashes are maximal, then the difference in half-times approaches 3.5-fold in some experiments. The differences in initial rates were considerably greater, and were more than 10-fold in some preparations (see Fig. 1). However, these rates could not be determined with accuracy. These results appear to be in contradiction with the results of Diner [16], who estimated equilibrium constants for the reactions $Q^-B \rightleftharpoons QB^-$ and $Q^-B^- \rightleftharpoons QB^{2-}$ of 15—20 and greater than 50, respectively. Since an equilibrium constant is the quotient of the forward and reverse rate constants $K = k_n/k_{-n}$, Diner's values suggest that the reaction $Q^-B^- \rightarrow QB^{2-}$ should be faster than the reaction $Q^-B \rightarrow QB^-$, unless there is a very large difference in the reverse rate constants for the two reactions.

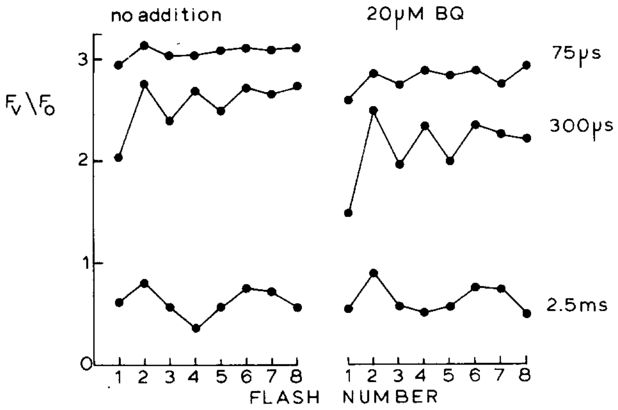


Fig. 3. Fluorescence measured at 75 μs, 300 μs and 2.5 ms after each of a series of saturating laser flashes given to dark-adapted chloroplasts in the presence or absence of benzoquinone (BQ). Protocol as described in legend in Fig. 1. Spinach chloroplasts were dark adapted in the presence or absence of 20 μM BQ (291 mV), and fluorescence measured at the times indicated after each of eight laser flashes (frequency : 1/s). Chlorophyll concentration 7.5 μg · ml^{-1}.

If we assume that the rate constants for the reverse reactions are similar, then our results would suggest that the value of $E_m(B/B^-)$ is at least 60 mV more positive than that of $E_m(B^-/B^{2-})$. This difference is in the direction previously suggested by Bendall [17], in a discussion of a possible mechanism for the two-charge accumulation system, and that computed by Bouges-Bocquet [18].

Decay of fluorescence as a function of flash number

Fig. 3 shows the fluorescence yield measured at 75 μs, 300 μs and 2.5 ms after each of a sequence of eight flashes given to spinach chloroplasts in the presence or absence of benzoquinone. In all experiments at E_h values less than 350 mV, the fluorescence yield measured at 50 μs after any number of actinic flashes following dark adaption, was similar within the error margin of the measurements. After attaining the same initial level, the rate of decay of the yield oscillated as a function of flash number with a marked periodicity of two, in line with the above model. The oscillation was more pronounced and in the same phase in the presence of benzoquinone, and was particularly apparent on the first two flashes under the latter conditions. This latter effect is unlikely to be due to the presence of a residual fraction of the secondary acceptor Q2 (E_m (pH 7) ≈ 390 mV) [19] at this potential (300 mV), since the maximal initial level of fluorescence was similar after each flash. At higher redox potentials the effect of oxidising Q2 before the flash could be readily observed (see below).

At times closer to the flash the biphasic oscillations became less marked. The oscillation was most pronounced at approximately the half-time for the reaction of $Q^-B \rightarrow QB^-$, as might be expected [3,4,9].

Effect of redox potential

We have made a preliminary study of the effect of redox potential (E_h) on

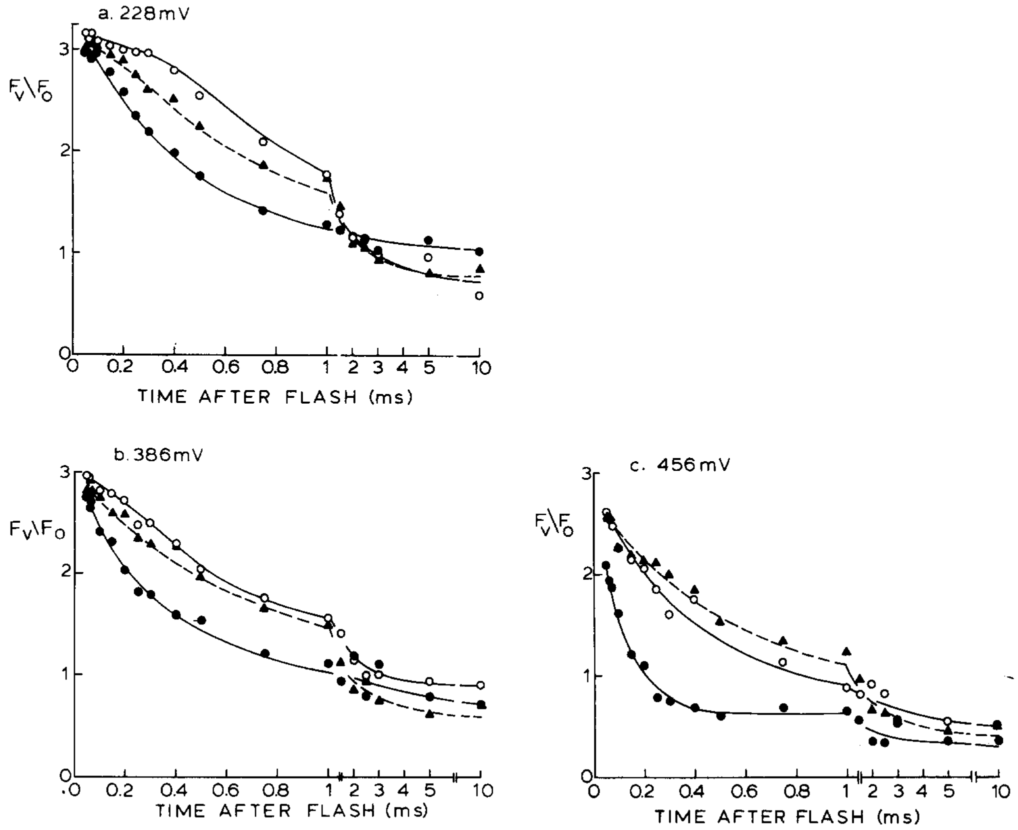


Fig. 4. Effect of redox potential on the decay of fluorescence following one, two or three saturating laser flashes given to dark-adapted chloroplasts. Protocol as described in legend to Fig. 1. Spinach chloroplasts were preincubated in the dark for 10 min in the presence of 20 μM benzoquinone; the potential was adjusted to the value indicated with aliquots of potassium ferri-/ferrocyanide and sodium ascorbate. Fluorescence was measured after flash 1 (●———●), 2 (○———○) and 3 (▲———▲) (frequency : 1/s) as described in legend to Fig. 3. Chlorophyll concentration 8.69 $\mu g \cdot ml^{-1}$.

the oscillations in the rate of oxidation of Q^-, and on the initial fluorescence yield. Fig. 4a–c shows the kinetics of decay at three values of E_h for the first three flashes. First-order plots of the decays after one or two flashes at an E_h of 291 mV showed major kinetic components of $t_{1/2} \approx 400$ μs (after flash 1) and $t_{1/2} \approx 700$ μs (after flash 2). At an E_h of 456 mV, a faster kinetic component of $t_{1/2} \approx 140$ μs accounted for most of the decay after the first flash, and the second flash now had a major decay component of $t_{1/2} \approx 400$ μs. The decay after a third flash was slower than that after the second and contained decay components of $t_{1/2} \approx 500$ μs and approx. 900 μs, with the slow component contributing the major share. We do not wish to lay too great an emphasis on the values for decay constants obtained from these data since the points show a significant scatter, and the error margin is considerable. In addition, the points at times closer to the flash contain contributions from the kinetics associated with events on the donor side [20], in particular following

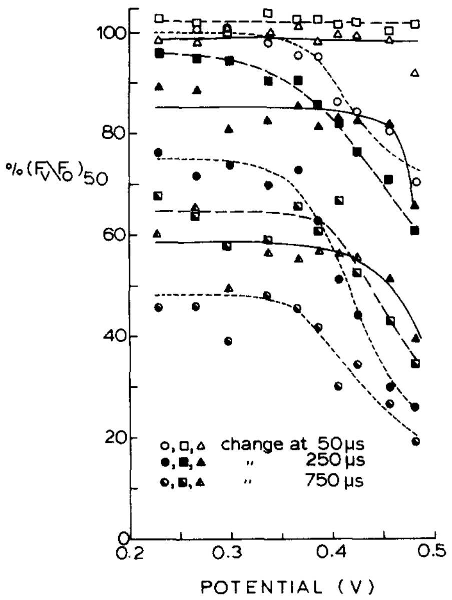


Fig. 5. Effect of redox potential on fluorescence at 50 μs, 250 μs and 750 μs after each of one, two or three saturating laser flashes given to dark-adapted chloroplasts. Conditions as described in legend to Fig. 3. Flash 1; circles, short-dashed lines: flash 2; squares, long dashed lines: flash 3; triangles, solid lines. Open symbols; 50 μs after flash: half-closed symbols; 750 μs after flash: closed symbols; 250 μs after flash. Vertical scale shows normalised variable fluorescence (F_V/F_0) expressed as a percentage of the value at 50 μs at each potential (average of this value for flashes 3—8).

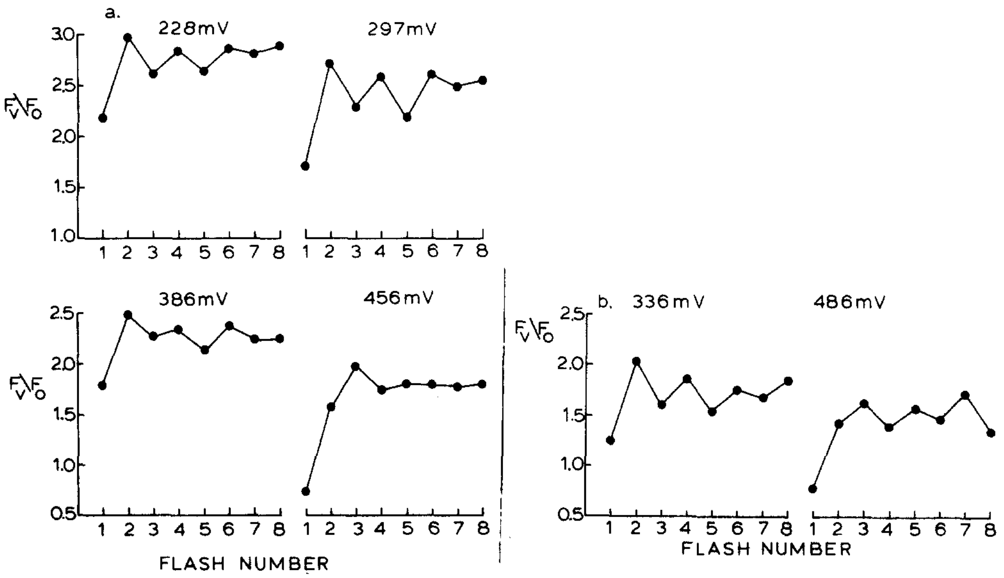


Fig. 6. Effect of redox potential on fluorescence measured after each of eight saturating laser flashes given to dark-adapted chloroplasts. (a) Conditions as described in legend to Fig. 4. Fluorescence was measured 300 μs after each of a sequence of eight laser flashes given to dark-adapted spinach chloroplasts (frequency : 1/s). Chlorophyll concentration 8.69 μg · ml^{-1}. (b) Protocol as described in legend to Fig. 4. Fluorescence measured at 250 μs after flash. Pigweed chloroplasts were used (conditions described in legend to Fig. 1). For the measurement at 486 mV, the chloroplasts were preincubated in the presence of 0.2 mM $K_3Fe(CN)_6$. Chlorophyll concentration 8.12 μg · ml^{-1}.

flash two at lower values of E_h, and also show a lag. However, the general pattern is clear, and this is brought out more clearly in Fig. 5 which shows the extent of the fluorescence yield remaining at different times after the first three flashes as a function of redox potential. Fig. 6a shows the pattern of oscillation as a function of flash number at several values of E_h, at 300 μs (or 250 μs, Fig. 6b) after the flash. Over the potential range in which benzoquinone provided useful mediation, the binary oscillation was fairly independent of redox potential (Figs. 4—6). Above 350 mV (Figs. 4c, 5 and 6) the effect of oxidation of Q2 [19] became apparent as a loss of fluorescence yield at 50 μs on the first flash only. As this effect titrated in, the binary oscillation was delayed by one period, and partly damped (Fig. 6).

We have not yet explored effects at the lower end (less than 150 mV) of the redox potential range in detail. However, the pattern of oscillations observed with normal chloroplasts was not greatly affected by preincubation with 2 mM sodium ascorbate ($E_{m7} \approx$ 60 mV) and 2 μM diaminodurine under aerobic conditions. We are at present extending our measurements to include full redox titrations under anaerobic conditions.

Possible mechanisms

In the mechanism of charge accumulation suggested by Bendall [17], the half-reduced B^- fails to reduce PQ because the redox potential of the couple B/B^- is higher than that of PQ/PQH_2. The couple B/B^{2-} can reduce PQ/PQH_2, and the formal relationship between E_m values requires that the couple B^-/B^{2-} is more reducing than B/B^{2-}. These relative values are in the directions suggested by our present results, but are opposite to those estimated by Diner [16]. In effect, Bendall's mechanism requires a thermodynamically stable semiquinone species; presumably this stabilisation would be achieved by a special protein environment. Such a stabilisation is consistent with the apparent stability of B^- observed spectrophotometrically [3,4], and required to account for the binary oscillations; however, such a stability could clearly be the result of kinetic rather than or as well as thermodynamic factors. We will use Bendall's mechanism as the basis for a further discussion of our results.

Relative redox potentials of the couples B/B^- and B^-/B^{2-}

As discussed above, the differences in half-times and initial rates, assuming similar reverse rate constants, gave a value for the difference in redox potential between the couples varying from 20 mV to greater than 60 mV, with B^-/B^{2-} being the more reducing couple. It seems possible that the difference might be even greater than the higher of these values, since the time course of Q^- oxidation after a second flash was clearly determined by tertiary effects rather than by simple first or second-order kinetics. We have not yet investigated the nature of these effects in detail. In order to explain the oscillations in terms of purely thermodynamic effects according to Bendall's scheme [17], the redox potential of B/B^- would have to be at least 30 mV more positive than the mid-potential of PQ/PQH_2 (80 mV) [21], and the redox potential of the couple B^-/B^{2-} would then be expected to have a potential more negative than 80 mV by the same amount, since the midpoint potentials of B/B^{2-} and PQ/PQH_2 are the same [17]. The redox potential difference between the two B couples estimated on this basis seems within the range that our results would suggest. However, it is clear that more data are needed before we can decide whether or not a purely thermodynamic mechanism can account for the binary oscillation.

The differences between the relative redox potentials suggested by our results, and those estimated rather indirectly by Diner [16] may be due to the fact that, contrary to our assumption, the back reaction rate constants are far from equal, or they may indicate a degree of imprecision in the values for concentrations of reactants and products derived by Diner in the estimation of equilibrium constants. Van Best and Duysens [22], using *Chlorella*, estimated that both couples (B/B^-, B^-/B^{2-}) must have equilibrium constants with respect to Q/Q^- greater than 5. It seems likely from their conditions that in the case of the couple B/B^-, the equilibrium constant with respect to the couple Q/QH was also greater than 5, since the value was determined with respect to centers in which approx. 70% of the acceptor was chemically reduced before the flash, and would therefore have equilibrated with a proton. The difference between the midpoint potentials of Q/Q^- and Q/QH is determined by the pK for dissociation of QH, and the pH of the phase in equilibrium with the couple. Neither of those values is known precisely for *Chlorella*. In spinach chloroplasts, $E_m(Q/Q^-) = -130$ mV and $E_{m7}(Q/QH) \approx 0$ [23]. An equilibrium constant $K > 5$ with respect to Q/QH at pH 7 would be equivalent to $K > 150$ with respect to Q/Q^-.

An alternative explanation for the discrepancy between our interpretation and that of Diner [16] may be sought in a consideration of the involvement of protons in the equilibria of the couple B^-/B^{2-}. Diner's measurements were made sometime after excitation when any protonation reaction would have finished, and one would expect that the equilibrium constant measured would be that for the reaction

$$Q^-B^- + 2\,H^+ \rightleftharpoons QBH_2$$

Diner [16] considered the possibility that the equilibrium constant for this reaction was much greater than that for the reaction leading to QB^{2-}. If this were the case, then the sequence of reactions after a second flash would be

$$Q^-B^- \underset{K_a}{\rightleftharpoons} QB^{2-};\quad QB^{2-} + 2\,H^+ \underset{K_b}{\rightleftharpoons} QBH_2$$

and it might be that the equilibrium constant, K_a, would be measured close to the flash (our result), and $K_a \cdot K_b$ after protonation (Diner's result). Although this mechanism appears to account rather well for the discrepancy, two points argue against it. Firstly, as shown by Diner and Joliot [11], the rate of electron transport from Q^- (as measured by the turnover of Photosystem II) does not increase as the pH is lowered. We have reached a similar conclusion using the more direct method of measurement reported above (Bowes, J.M., unpublished data). In fact, as previously observed [11], the rate became slower when the pH was lower. It seems that the rate of the reaction is not determined by the proton concentration of the bulk aqueous phase, as might have been expected if K_a were less than 1 as envisaged above. Secondly, no simple linear series of reactions can account for the curious lag in the onset of oxidation of Q^- following the second flash. In all simple schemes, the rate of loss of Q^-, given by

$$\frac{d[Q^-]}{dt} = k_a[Q^-B^-] - k_{-a}[QB^{2-}] ,$$

must be maximal initially. The kinetic behavior is rather that of an autocatalytic process, but it seems at present premature to speculate as to what this means in terms of mechanism.

We hope that having identified a difference in the kinetics of their two reactions, we will be able to estimate the concentrations of B and B^- as a function of redox potential, and hence arrive at an unambiguous value for the midpotentials of the couples.

The involvement of Q2 in the acceptor-side reactions

We have previously suggested that at redox potentials above its equilibrium midpoint potential ($E_m \approx 400$ mV varying by —60 mV/pH) a second acceptor, Q2, is available to rapidly accept electrons from Q, the normal primary acceptor [19]. This interpretation of our previous results seems well supported by the present work. In particular, the delay of one period in the binary oscillations, and the partial damping suggest that Q2 has a capacity for between 0.5 and 1 electron, and that the electron which reduces Q2 is not available to the rest of the chain. We had previously discussed the possibility of a reaction between reduced Q2 and the couple B^-/B^{2-} in terms of the possible high redox potential of this couple as suggested by Diner's [16] equilibrium constants. Our present results would seem to preclude such an interaction, and would suggest that B^-/B^{2-} is more reducing than B/B^-, as discussed above. The delayed oscillation suggests that $Q2^-$ cannot reduce B either, but the results do not throw any further light on the nature or role of the component Q2. It is worth noting that the faster decay kinetics seen after the first flash at high redox potential (Fig. 4c) include a major component of $t_{1/2} \approx 140$ µs which on extrapolation to zero time accounts for most of the decay from a level similar to that measured at 50 µs after a second flash. This implies a rate of electron transfer from Q^- to Q2 considerably slower than the rate ($t_{1/2} < 5$ µs) we had previously estimated [19]. It may be that a much faster component of the decay has been 'lost' in the complex kinetics at times closer to the flash than our first point at 50 µs. Alternatively, we may previously have estimated too fast a time for the reaction by which Q^- reduces Q2. While this manuscript was in preparation, Eckert et al. [24] reported measurements of a turnover time of 100 µs for Photosystem II for chloroplasts poised at high redox potential. However, this value was obtained under repetitive flash conditions, and we are unsure how it may relate to our present results obtained with dark-adapted chloroplasts. If the two values are comparable, it seems highly unlikely that the turnover time measured by Eckert et al. [24] can be accounted for in terms of a second primary acceptor operating in parallel with Q under conditions in which the normal primary acceptor is reduced, as these authors suggest. Rather our results would indicate that electron transfer occurs from Q to a secondary acceptor which becomes oxidised only at $E_h > 350$ mV. We are at present improving our apparatus so as to be able to resolve the kinetics closer to the flash, and with a better reproducibility in order to test these points.

Acknowledgements

We should like to thank Gerry Tierney for design, construction and maintenance of the microprocessor-controlled data sampler used in this research, and Cathy Ditto for providing pigweed chloroplasts. This research was funded by a National Science Foundation grant (No. PCM 7816574) to A.R.C.

References

1 Bouges-Bocquet, B. (1973) Biochim. Biophys. Acta 314, 250—256
2 Velthuys, B.R. and Amesz, J. (1974) Biochim. Biophys. Acta 333, 85—94
3 Pulles, M.P.J., van Gorkom, H.J. and Gerben Willemsen, J. (1976) Biochim. Biophys. Acta 449, 536—540
4 Mathis, P. and Haveman, J. (1977) Biochim. Biophys. Acta 461, 167—181
5 Bennoun, P. and Joliot, A. (1969) Biochim. Biophys. Acta 189, 88—94
6 Yamashita, T. and Butler, W.L. (1968) Plant Physiol. 43, 1978—1986
7 Forbush, B., Kok, B. and McGloin, M. (1971) Photochem. Photobiol. 14, 307—321
8 Duysens, L.N.M. and Sweers, H.E. (1953) Studies on Microalgae and Photosynthetic Bacteria, pp. 353—372, University of Tokyo Press, Tokyo
9 Forbush, B. and Kok, B. (1968) Biochim. Biophys. Acta 162, 243—253
10 Zankel, K.L. (1973) Biochim. Biophys. Acta 325, 138—148
11 Diner, B.A. and Joliot, P. (1976) Biochim. Biophys. Acta 423, 479—498
12 Bowes, J.M. and Crofts, A.R. (1978) Z. Naturforsch. 33c, 271—275
13 Arntzen, C.J., Ditto, C.L. and Brewer, P.E. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 278—282
14 Joliot, A. (1974) in Proc. 3rd Congr. on Photosynthesis Research, Rehovot, Vol. 1, pp. 315—322, Elsevier, Amsterdam
15 Wollman, F.-A. (1978) Biochim. Biophys. Acta 503, 263—273
16 Diner, B.A. (1977) Biochim. Biophys. Acta 460, 247—258
17 Bendall, D.S. (1977) in Plant Biochemistry II (Northcote, D.H., ed.), M.T.P. International Review of Biochemistry, Vol. 13, pp. 41—78, University Park Press, Baltimore, London and Tokyo
18 Bouges-Bocquet, B. (1975) in Proc. 3rd Congr. on Photosynthesis Research, Revohot, 1974 (Avron, M., ed.), Vol. 1, pp. 579—588, Elsevier, Amsterdam
19 Bowes, J.M., Crofts, A.R. and Itoh, S. (1979) Biochim. Biophys. Acta 547, 320—335
20 Duysens, L.N.M., den Haan, G.A. and van Best, J.A. (1975) in Proc. 3rd Congr. on Photosynthesis Research (Avron, M., ed.), Vol. 1, pp. 1—12, Elsevier, Amsterdam
21 Okayama, S. (1976) Biochim. Biophys. Acta 440, 331—336
22 Van Best, J.A. and Duysens, L.N.M. (1977) Biochim. Biophys. Acta 459, 187—206
23 Knaff, D.B. (1977) FEBS Lett. 60, 331—335
24 Eckert, H.J., Buchwald, H.E. and Renger, G. (1979) FEBS Lett. 103, 291—295