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# Electron transfer to P-680$^+$ in active and inhibited Photosystem II fractions from higher plants

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**We have studied the kinetics of P-680$^+$ reduction in PS II fractions which were prepared with high rates of oxygen evolution from pea thylakoids. In noninhibited fractions, about two-thirds of the photooxidized P-680 was reduced in less than 2 μs (the minimum time resolution of the instrument), and phases with lifetimes of 5–40 μs may be linked with electron donation in PS II. After inhibition of electron donation to P-680$^+$, rereduction kinetics were biphasic with lifetimes of 90–150 μs and 600–900 μs. We argue that the faster component may represent a back-reaction from the state [$D_1^+$ P-680$^+$ ... $Q^-B^-$], and that the slower component may represent a back-reaction from the state ($D_1$//P680$^+Q^-$B]. From experiments with low concentrations of water analogues we propose that the complex multi-phasic kinetics of electron donation from $D_1$ to P-680$^+$ could be a reflection of the dynamic properties of the water binding site.**

## Introduction

The absorption of light energy by PS II in plants and cyanobacteria results in the photooxidation of the primary electron donor, P-680. This species is so termed because of the large bleaching observed at 680 nm which is probably due to the oxidation of a specialised chlorophyll *a* [1]. The light-energy trapped in the oxidized chlorophyll (P-680$^+$) provides the oxidizing power for the eventual dehydrogenation of water and release of oxygen.

The initial measurements of the bleaching at 680 nm suggested that in higher plant chloroplasts, P-680$^+$ reduction occurred with biphasic kinetics of half-times of 35 and 200 μs [2,3]. Since then, improvements in the time resolution of instrumentation has led to the detection of other faster phases in the reduction kinetics, with half-times of 20–30 ns [1,4,5], 50 ns [4,5], 200–300 ns [4,5] and 2–5 μs [6,7]. A broad and weak absorption peak around 820 nm, which is probably due to the Chl $a^+$ cation, can also be used to monitor the kinetics of P-680$^+$ reduction, with the advantage that the measuring beam is not absorbed by the light-harvesting pigments. Van Best and Mathis [1] were the first to observe a 30 ns electron donation to P-680$^+$ by using this measuring wavelength to probe dark-adapted chloroplasts. Only the first flash was used in their experiment, and ferricyanide was present to oxidize P-700$^+$ in PS I which also shows a transient absorption change at 820 nm. They observed that inhibition of oxygen

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Abbreviations: B, secondary two-electron acceptor in PS II; Chl, chlorophyll; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; $D_1$, donor to P-680$^+$; Mes, 4-morpholineethanesulfonic acid; PS I, Photosystem I ; PS II, Photosystem II; P-680$^+$, oxidized primary donor of PS II; P-700$^+$, oxidized primary donor of PS I; Q, first stable electron acceptor in PS II.

evolution removed the 30 ns kinetics, and that P-680$^+$ reduction occurred mainly on a microsecond timescale.

Eckert et al [4], and Brettel and Witt [5], who used 680 and 836 nm, respectively, as the measuring wavelengths, have recently observed several nanosecond phases in the reduction of P-680$^+$ under repetitive flash-illumination. The improved signal-to-noise ratios of recent measurements compared to those of Van Best and Mathis [8,9] has allowed a study of the flash-number dependency of P-680$^+$ reduction kinetics. Brettel and co-workers used an 824 nm measuring wavelength for these experiments, and studied an oxygen-evolving PS II fraction from a cyanobacterium (*Synechococcus* sp.) as well as higher plant thylakoids [8,9]. They proposed that the heterogeneity observed in the nanosecond kinetics with repetitive flash illumination could be explained by assigning the different phases to PS II units with different 'S-states' [10], that is the number of oxidizing equivalents stored in the oxygen-evolving complex. They proposed that in the S-states $S_0$ and $S_1$ (where the subscript refers to the number of stored oxidizing equivalents), a 20–30 ns phase in the P-680$^+$ reduction occurred. Slower phases (50 and 300 ns) were correlated with the S-states $S_2$ and $S_3$.

Bouges-Bocquet [11] predicted an S-state dependence for electron donation to P-680$^+$ based on the model of two parallel donors to P-680$^+$. However, Brettel and co-workers have proposed that electron donation is slower in the higher S-states because of an interaction between the electron and a positive hole stored in the water-splitting complex. This model requires that the release of protons from the system should be in the pattern 1, 0, 1, 2, from the S-states $S_0$, $S_1$, $S_2$, $S_3$, respectively [12].

Thus, recent developments in instrumentation [4,5,7,8] and in the purification of PS II with retained oxygen-evolution activity [13] have produced a much clearer picture of electron transfer from water through to P-680$^+$. However, the presence of complex microsecond phases in the kinetics of P-680$^+$ reduction has largely remained unexplained. Conjeaud et al. [14] and, more recently, Telfer et al. [15] have estimated that less than 20% of the P-680$^+$ is reduced on a microsecond timescale. They used an 820 nm measuring beam and averaged the result of only a few laser flashes spaced several seconds apart. Other measurements with chloroplasts and oxygen-evolving PS II fractions were carried out with repetitive flash illumination and with 820 and 680 nm measuring wavelengths. Between 30 and 50% of the P-680$^+$ reduction occurred on a microsecond timescale [4–7,16].

These slower phases in the reduction of P-680$^+$ must also be studied in detail if we are to obtain a full understanding of electron-transfer events in PS II. Furthermore, the relationship between P-680$^+$ reduction kinetics and the final activity of the water-splitting system appears to be very strong [8,9], and a study of this relationship may give insight into the poorly understood mechanism of oxygen evolution.

The kinetics of P-680$^+$ are clearly very complex, and many phases can be distinguished on the nanosecond and microsecond timescales. In contrast, the kinetics of reduction of P-700$^+$ by its donor plastocyanin are much simpler [17].

In this paper we describe P-680$^+$ reduction kinetics in a PS II preparation with very high rates of oxygen evolution. We propose that both nanosecond and microsecond phases in the reduction kinetics can arise from the same donor to P-680$^+$ ($D_1$).

A fast back reaction between P-680$^+$ and $Q^-$ in PS II can also be detected on a microsecond time-scale with a half-time for the reduction of about 80 $\mu$s [18]. We have observed similar kinetics in samples where oxygen evolution has been inhibited, but we find much slower kinetics ($t_{1/2} \simeq$ 300 $\mu$s) when the donor $D_1$ has also been inhibited.

## Materials and Methods

Peas (*Pisum sativum*, variety Feltham First) were grown in vermiculite under fluorescent and tungsten filament lamps at a light-intensity of approx. 20 W · m$^{-2}$. Chloroplasts were prepared by the method of Nakatani and Barber [19] except that 5 mM ascorbate (disodium salt) was added to the grinding medium. Detergent fractionation was performed as described in Ref. 16, a method which is based on that reported originally by Berthold et al. [20]. This procedure allows the retention of full

oxygen-evolution activity in the final PS II fraction.

For storage at 77 K, the PS II fractions were suspended in 20 mM Mes/5 mM $MgCl_2$/20% glycerol (pH 6.3). The standard assay medium for oxygen evolution and optical experiments was 40 mM Mes/5 mM $MgCl_2$/10% glycerol (pH 6.0). Oxygen evolution was measured in a Clark-type oxygen electrode at 20°C. Potassium ferricyanide (2 mM) and 2,6-dimethylbenzoquinone (2 mM) were used as electron acceptors in both optical measurements and oxygen-evolution assays.

Absorption changes at 820 nm were measured at room temperature in a 1 cm path-length cuvette. A purpose-built single-beam spectrophotometer was used. A type PIN 10-D photodiode (United Detector Technology, CA, U.S.A.) was used as the detector, coupled to a low-noise differential amplifier (EG/G Princeton Applied Research, Model 113). The resolution time limit of the instrument was about 1.5–2 μs. The measuring beam was provided by a 250 W tungsten filament lamp powered by a Coutant direct current supply (Model ASC3000 PC). The beam was passed through a 780 nm long-pass filter (Schott RG780), and then through the sample cuvette, and focussed on the slit of a monochromator (Applied Photophysics model 7500) set at 820 nm. Signals were recorded using a Datalab 920 transient recorder, and averaged with a Datalab 4000B signal averager. Subtraction of artefacts due to chlorophyll fluorescence was achieved by the accumulation of an equal number of recordings with the measuring beam switched off. Excitation pulses were provided by Chromatix tunable dye laser using Rhodamine 640 (Exciton Chemical, OH, USA) as the dye. Pulses with a full-width at half-maximum intensity of about 600 ns were obtained at about 660 nm.

## Results

The detergent treatment originally described by Berthold et al. [20] was at pH 7.5, and when we first prepared PS II in this way low rates of oxygen evolution were obtained (about 100–200 μmol $O_2$ per mg Chl/h). With these fractions virtually all the $P\text{-}680^+$ decayed on a microsecond time-scale with only about a 20% increase in the amplitude of the signal after the addition of hydroxylamine [6,14]. The microsecond phases were dominated by a component with slow kinetics (lifetime, approx. 150 μs). The addition of artificial donors to PS II reduced the extent of the slow phase and produced faster kinetics.

The fractionation at pH 6.3 which we have used seems to preserve most if not all of the oxygen-evolution activity present in the isolated chloroplasts [16]. The extent of the microsecond-detected phases was much smaller, and also the kinetics of the decay were faster than samples obtained by pH 7.5 fractionation. The effect of artificial electron donors was small.

In a previous paper [16] we reported the detection of significant microsecond phases in the reduction of $P\text{-}680^+$ in oxygen-evolving PS II fractions from spinach. In this report we used PS II fractions derived from peas which were grown under more rigorously controlled lighting conditions. We found that in general the extent of the microsecond phases was smaller (30–40% of the total signal compared to 40–50% in spinach). This difference did not correlate with lower rates of oxygen evolution, as in both spinach and pea material the activity was found to be between 700 and 1200 μmol $O_2$ per mg Chl/h at saturating light intensities.

Fig. 1a shows a typical experiment to investigate the kinetics of $P\text{-}680^+$ reduction in a pea PS II fraction which had a rate of oxygen evolution of 780 μmol $O_2$ per mg Chl/h. The decay is multiphasic and probably includes a contribution from $P\text{-}700^+$. Brettel and Witt [5] reported that $P\text{-}700^+$ decays with a half-life of about 30 ms under these conditions, and we estimate from separate measurements [16] that about 8% of the signal shown in Fig. 1a is representative of $P\text{-}700^+$, and that this fraction does not decay appreciably in 1 ms.

An accurate determination of the life-times of the remaining components is not feasible because of signal-to-noise considerations, although fast (life-time, 5–40 μs) and slower (life-time, 200–300 μs) components can be discriminated.

Since the minimum time resolution of the instrument was about 2 μs, the nanosecond components in the $P\text{-}680^+$ reduction kinetics could not be detected. The extent of the nanosecond phases was estimated by the addition of hydroxylamine (2

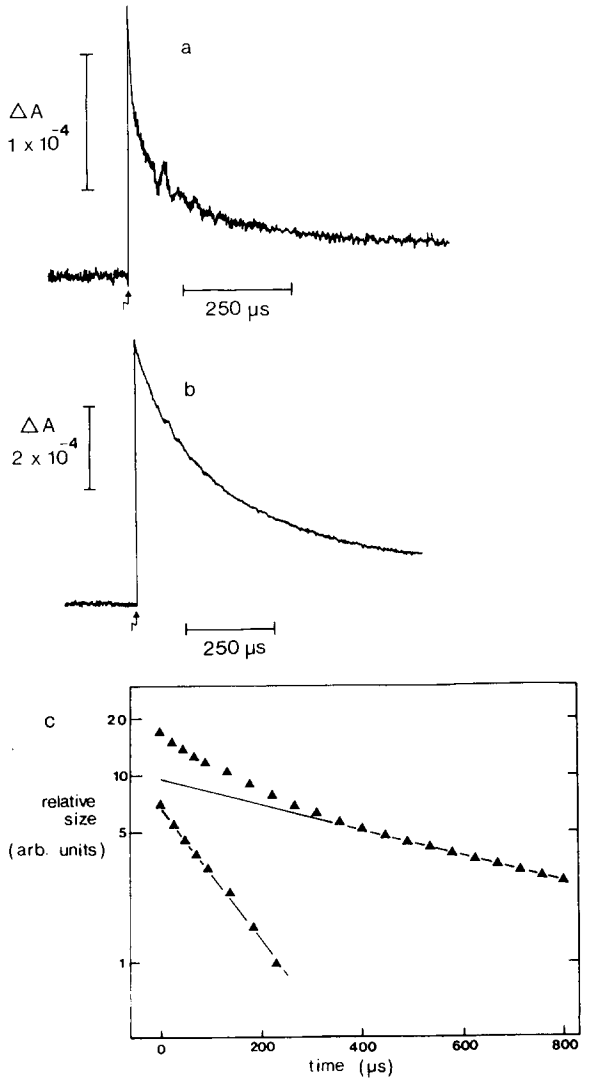


Fig. 1. (a) 820 nm absorption changes in an active PS II preparation from pea thylakoids suspended at pH 6 in the standard assay medium. Electron acceptors were added as described in the materials and methods section. 64 flashes were averaged at a frequency of 5 Hz. Chlorophyll concentration was 29 μg/ml. (b) As in (a), but with the addition of hydroxylamine (2 mM) followed by 30 s illumination with a projector. (c) This shows a semi-logarithmic plot of the data shown in (b). Two phases appear to be present (see text).

mM), as shown in Fig. 1b and c) [1,6,14]. In this experiment, the sample was illuminated for 30 s with a 1000 W projector after the addition of hydroxylamine, a treatment which inhibits water oxidation and electron transfer from $D_1$ to P-680$^+$ [16,21]. The microsecond-detected P-680$^+$ signal was larger after inhibition treatment (35% was detected prior to inhibition), and the kinetics of the decay were slower with a major phase (55%) of lifetime 600–700 μs and a smaller component (45%) of life-time 120 μs (Fig. 1c).

If hydroxylamine (2 mM) is added without the illumination of the sample, then water oxidation is blocked, but it acts as an alternative donor of electrons to PS II and a strong pH-dependence is observed in the kinetics of P-680$^+$ reduction under these conditions [22,23]. In agreement with these reports we found that at pH 6, P-680$^+$ was reduced with a half-time of 10–20 μs after the addition of hydroxylamine, but we also observed that these fast kinetics were gradually replaced by much slower phases in the decay similar to those shown in Fig. 1b and c. This effect seemed to increase with prolonged incubation of the sample with hydroxylamine as well as with the number of laser flashes used for the measurements.

The inhibitory action of hydroxylamine would therefore seem to involve at least two effects [21]. In one case it acts as an electron donor to PS II, possibly competing with water for this role [24] and in the other case, after strong illumination or long incubation times, it seems to inactivate the donor side of PS II. In accordance with these conclusions we found that the EPR-detected signals, signal II and signal $II_{LT}$ were greatly reduced after the illumination of hydroxylamine-treated samples. The light-induced low-temperature component termed signal $II_{LT}$ has been associated with the oxidation of the first donor to P-680$^+$ [25]. This treatment was also found to release $Mn^{2+}$ into an EPR-detectable state.

Electron donation to P-680$^+$ seems to be highly sensitive to the pH of the medium, whereas the kinetics of the back-reaction in inhibited samples is relatively pH-independent [22,23,26]. Table I shows the pH-dependence of the decay kinetics of P-680$^+$ using the half-time as an approximate indicator of the multi-phasic decay detected on a microsecond time-scale. Prior to inhibition, there is a strong pH dependence in the microsecond-detected kinetics, but illumination of hydroxylamine-treated samples appears to remove the pH-dependence of P-680$^+$ reduction completely.

There appear to be two factors which determine the half-time for P-680$^+$ reduction in non-in-

TABLE I

pH-DEPENDENCE OF THE HALF-TIMES FOR P-680$^+$ REDUCTION DETECTED ON A MICROSECOND TIME-SCALE IN CONTROL FRACTIONS AND AFTER INHIBITION WITH HYDROXYLAMINE (2 mM) AND 30 s ILLUMINATION.

64 measurements were averaged, at a flash frequency of 5 Hz, the first 32 measurements were rejected. The figures in brackets give the approximate fraction of P-680$^+$ detected after 2 μs.

| pH | Half-time (μs) | |
|---|---|---|
| | Control | Inhibited |
| 5.0 | 60 (0.57) | 290 |
| 5.5 | 58 (0.44) | 280 |
| 6.0 | 52 (0.41) | 300 |
| 6.5 | 37 (0.40) | 200 |
| 7.0 | 16 (0.41) | 260 |
| 7.5 | 77 (0.54) | 350 |
| 8.0 | 90 (1.00) | 220 |
| 8.5 | 75 (1.00) | 250 |

hibited samples. Firstly, as the pH is made more alkaline, the rate of electron transfer from $D_1$ to P-680$^+$ is increased [23]. Secondly, oxygen evolution is inhibited above pH 7.5 and below pH 5.5 in this type of PS-II-detergent fraction, and therefore with repetitive flashes an increasing proportion of a slow back-reaction is observed at these extremes of pH, since only one electron is available from the donors. Fig. 2 shows the pH-dependence of oxygen evolution in a pea PS II fraction. The rates were found to be much less stable above pH 7, with illumination causing a rapid inhibition at pH 8.

Above pH 8, oxygen evolution is completely inhibited, and a slow back-reaction is observed after the second flash given to a dark-adapted sample [16] with the full extent of the P-680$^+$ absorption change detected on a microsecond time-scale. We have used these various conditions to investigate the kinetics of electron return from the acceptor side of PS II, and Fig. 3 compares the P-680$^+$ reduction kinetics after different inhibition treatments.

Fig. 3a shows P-680$^+$ reduction at pH 8.5 with repetitive flashes spaced 200 ms apart and Fig. 3b shows the reduction kinetics in the same sample after 30 s continuous illumination in the presence

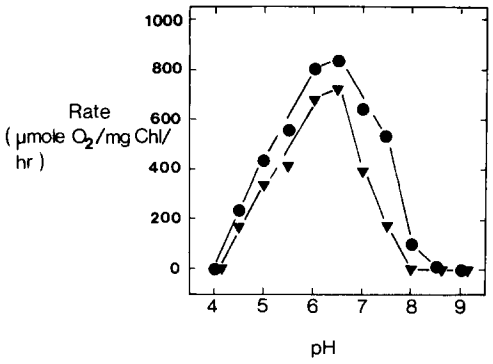


Fig. 2. pH-dependence of the rates of oxygen evolution in the PS II preparation. The rates at time zero (○) and 30 s (▽) are shown.

of hydroxylamine (2 mM). In Fig. 3a the decay of P-680$^+$ was almost monophasic with a lifetime of about 100 μs. A minor slow phase (18%) was also present with a life-time estimated at 800 μs. After the hydroxylamine treatment the extent of the slow component increased to 65% of the total decay although a 100 μs phase could still be detected. The life-times of the two components are almost identical with those shown in Fig. 1b which were measured at pH 6, but some variability is observed in the relative amplitudes of the two phases.

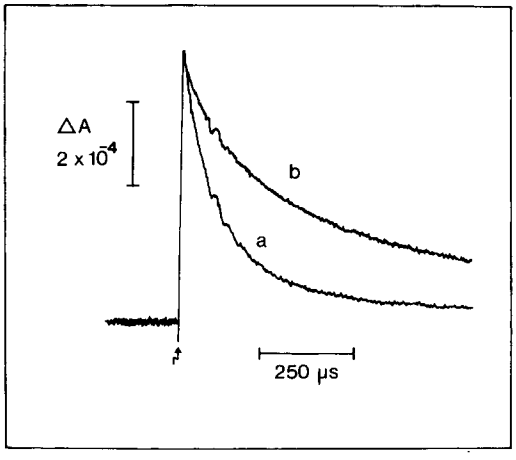


Fig. 3. (a) 820 nm absorption changes in a PS II fraction suspended at pH 8.5, 64 flashes were averaged; the first eight flashes in the train were not recorded. Chlorophyll concentration was 29 μg/ml. (b) As in (a), but after the addition of hydroxylamine (2 mM) followed by 30 s illumination with a projector.

We considered that the difference in the back-reaction kinetics after different inhibition treatments could be a reflection of the presence of a donor to P-680$^+$, and in order to test this hypothesis we studied the effect of DCMU, an inhibitor which blocks between Q and B. If P-680$^+$ is reduced by a donor after the first flash, then as $Q^-$ is relatively long-lived, subsequent excitations should give rise to the [P-680$^+$, pheophytin$^-$] pair which decays in a back-reaction of life-time about 4 ns [27]. Thus we should detect no signal with repetitive illumination if an effective donor to P-680$^+$ exists.

Figs. 4 and 5 show the effect of the addition of DCMU on the kinetics of the back-reaction after pH 8.5 and hydroxylamine treatment, respectively. As shown in Fig. 4, only a very small absorption change could be detected after the addition of DCMU, suggesting that $D_1$ is functional after pH 8.5 treatment, whereas after hydroxylamine treatment a large proportion of the absorption change was still observed, with predominantly the very slow kinetics of a life-time of 700 $\mu$s (Fig. 5b). The loss of the one phase after DCMU addition may

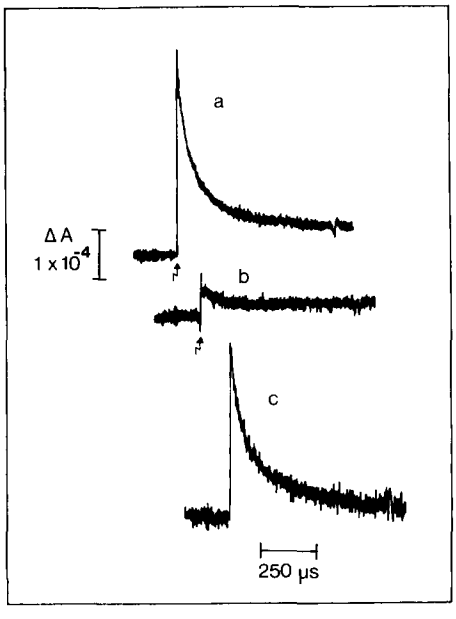


Fig. 4. (a) 820 nm absorption changes in a PS II fraction suspended at pH 8.5; 64 flashes were averaged, the first eight flashes were not recorded. Chlorophyll concentration was 16 $\mu$g/ml. (b) As in (a) but with DCMU (10 $\mu$M). (c) Difference (a – b).

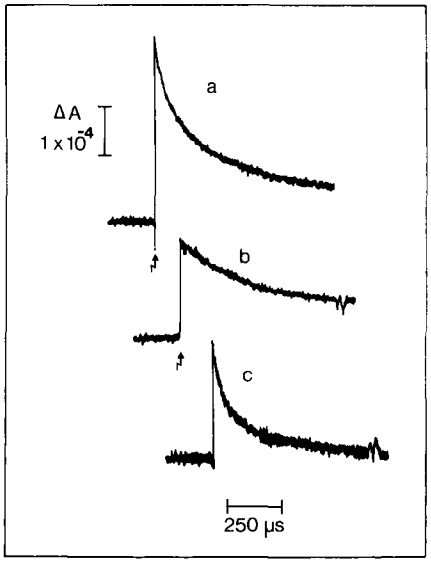


Fig. 5. (a) 820 nm absorption changes in a sample inhibited with hydroxylamine (2 mM) and light (30 s illumination); 64 flashes were averaged. Chl concentration was 16 $\mu$g/ml, (b) As in (a) but with DCMU (10 $\mu$M), (c) Difference (a – b).

be the result of donation from $D_1$ in those centres where the donor has not been removed by hydroxylamine treatment. When the sample was dark-adapted for a few minutes after the hydroxylamine-plus-light treatment, the first flash gave rise to a fast component of life-time of about 20 $\mu$s (49%), with a much slower phase (51%) of life-time of 500 $\mu$s. The 20 $\mu$s phase is typical of donation from $D_1$ in the presence of hydroxylamine [23].

The fast (5–40 $\mu$s) phases shown in Fig. 1a seem to arise from electron donation to P-680$^+$, although these phases represent only about 20% of the total decay which mostly occurs on a nanosecond time-scale. In the presence of very low concentrations of hydroxylamine, we have found that under certain conditions almost all of the P-680$^+$ was rereduced on a microsecond rather than nanosecond timescale. This could occur without any inhibition of the water-splitting system.

Fig. 6a shows the extent and kinetics of the decay of the P-680$^+$ absorption change after the first flash given to a PS II fraction which was incubated with 50 $\mu$M hydroxylamine for 5 min in the dark. At this concentration, hydroxylamine did not show any effect on the steady-state rate of oxygen evolution.

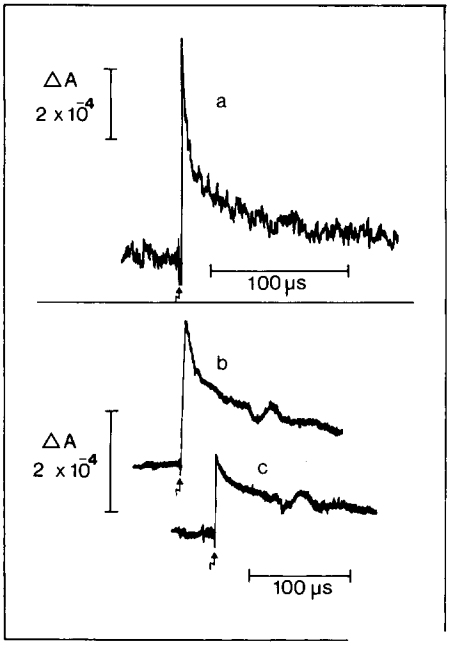


Fig. 6. (a) 820 nm absorption changes after the first flash given to a PS II fraction incubated for 5 min in the dark with 50 μM hydroxylamine. Chlorophyll concentration was 29 μg/ml. The result of one flash is shown; the fluorescence artefact (downward transient) was not subtracted in this case to maintain the signal to noise ratio. (b) As in (a) but the average of 32 flashes at a frequency of 5 Hz. The first 32 flashes were not recorded. (c) As in (b) but at a flash frequency of 25 Hz.

Others workers have shown that at these low levels hydroxylamine appears to bind at the site for water in the oxygen-evolving complex, and is oxidised after the first flash given to the sample [24,28]. From Fig. 6a it would appear that the presence of hydroxylamine at the water-binding site caused a change in the kinetics of P-680$^+$ reduction from the nanosecond to the microsecond time-scale. Virtually all of the P-680$^+$ decayed between 2 and 20 μs after the first flash, but subsequent flashes reduced the amplitude of the microsecond phases to control levels. Fig. 6b shows the P-680$^+$ absorption changes in the same sample with 32 measurements averaged at a frequency of 5 Hz. When laser pulses were provided at a frequency of 25 Hz, the amplitude of the microsecond-detected phases was slightly smaller (Fig. 6c). This flash-frequency effect may be a reflection of the rate at which hydroxylamine in solution can bind to the water-splitting complex.

We studied the effect of other water analogues on P-680$^+$ reduction kinetics in active PS II fractions. We found that it was only those analogues which bound strongly to the water-splitting system (such as hydroxylamine, hydrazine, hydroxylamine-*o*-sulfonate) which had any effect on the microsecond-detected amplitude [24]. Hydroxylamine, which binds most strongly, showed the largest effect on the microsecond-detected amplitude. This result does not rule out the possibility that P-680$^+$ reduction on a nanosecond time-scale could be affected by water analogues which bind less strongly than hydroxylamine.

## Discussion

In highly active pea PS II fractions we have estimated that about two thirds of the P-680$^+$ population is reduced on a submicrosecond time-scale. Since the width of the laser pulses we used was about 600 ns, it could be argued that the remaining one-third which was reduced on a microsecond time-scale was the result of multiple turnovers of the PS II reaction centre. This possibility can probably be ruled out, since we have recently obtained similar results for the amplitude and decay kinetics of the microsecond components (in spinach PS II fractions) using a 3 ns laser pulse (Brettel, K., Ford, R.C., Schlodder, E., Atkinson, Y.E., Witt, H.T. and Evans, M.C.W. (1985), to be published in FEBS Lett.).

Since $D_1^+$ appears to be reduced in 50–1000 μs [29], and $Q^-$ is reoxidized with half times of 140 or 200–400 μs [35], then, in accordance with current models of the PS II reaction centre [11], a double turnover in less than one microsecond would leave an electron on each of the acceptors pheophytin and Q. The [pheophytin$^-$, P-680$^+$] pair has a very short lifetime [27], and should not be detected by our instrument.

Several explanations for the presence of microsecond phases in the reduction of P-680$^+$ have been put forward, in particular that these components represent centres where oxygen evolution and $D_1$ are not functional because of damage during preparation [8,14, 15].

In this paper, we have shown that microsecond reduction kinetics can occur in place of nanosecond phases without any permanent inhibition of

oxygen evolution. The reversible change between nanosecond and microsecond kinetics seems to be rapid, and may depend on the rate at which hydroxylamine, at a concentration of 50 $\mu$M, can bind to the water-splitting site. In this case, it seems unlikely that $D_1$ is damaged, but rather that the functioning of the donor is modified by the presence of hydroxylamine in the oxygen-evolving complex.

Radmer has proposed that hydroxylamine is bound tightly to the water-splitting complex because the molecule is able to bind to two sites in a concerted fashion, thereby greatly reducing the rate of dissociation compared to water [24]. There is a strong resemblance between one hydroxylamine molecule and two closely spaced water molecules. Thus, Radmer has proposed that two sites for water binding exist, located about 0.15 nm apart. It may be reasonable to propose that the two hypothetical sites do not normally approach so closely, and that the binding of hydroxylamine could cause a change in the conformation of the region. This change could also affect the functioning of $D_1$, giving rise to the 10- to 100-fold slower reduction kinetics for P-680$^+$. Changes in the conformation of the water-splitting complex may also be induced by other treatments such as Tris-washing; cholate treatment [23]; salt-washing [30] and pH 8 treatment [16], but these treatments are largely irreversible, suggesting that more drastic changes have occurred. These treatments inhibit water oxidation, but also allow $D_1$ to transfer one electron.

We would suggest that the microsecond phases in the reduction of P-680$^+$ in active and in inhibited PS II fractions are probably due to the same donor, $D_1$, which gives rise to the nanosecond kinetics. This donor would be identical to Z [29,31,32], which has been characterized by the rise and decay kinetics of signal II at room temperature. In Tris-treated chloroplasts the rise of signal II correlates with the decay of P-680$^+$ [23,31], whilst in non-inhibited systems, the decay of signal II (signal $II_{vf}$) seems to reflect electron donation from the S-states [29,32].

A simple model for the donor side of PS II would therefore seem to involve a single donor, $D_1$, between P-680 and the S-states.

A study of the S-state dependence of the microsecond components may help to clarify the relationship between $D_1$ and the oxygen-evolving complex.

Previously, the half-time for the back-reaction between P-680$^+$ and $Q^-$ has been reported as 80–150 $\mu$s in chloroplast and higher-plant PS II preparations [18,23,26]. In cyanobacterial PS II preparations, however, the back-reaction appears to be much slower, with a half-time of about 800 $\mu$s [23]. No explanation for this difference compared to plant material has been advanced. We have also measured the back reaction between P-680$^+$ and $Q^-$ in PS II detergent particles prepared from the F54-14 mutant of the green alga *Chlamydomonas reinhardtii*. In these particles, the back-reaction is also much slower than in higher plant material with a half-time of 500 $\mu$s.

We would propose that the fast 80–150 $\mu$s phase represents the back reaction from the state $[D_1^+ \ P680^+ \dots Q^-B^-]$ to the more stable state, $[D_1^+ \ P680 \dots QB^-]$. The presence of an electron on B and a positive charge on $D_1$ could increase the rate of electron return from $Q^-$ to P680$^+$. In the cyanobacterial PS II preparations a large proportion of the electron transport rate is DCMU-insensitive [33]. In the *C. reinhardtii* PS II particles, DCMU-insensitive electron transport is also predominant [34]. In these PS II preparations, the interaction between Q and B may be weaker than in higher plants, and we would propose that this factor could explain the slower back-reaction from $Q^-$ to P-680$^+$.

In higher plant PS II preparations, the addition of DCMU usually abolishes the overall electron transport and any signals due to P-680$^+$ on a microsecond time-scale. After the inhibition of electron transfer from $D_1$ to P-680$^+$, we have shown that it is possible to observe the production and recombination of the P-680$^+$ $Q^-$ pair with repetitive illumination. Under these conditions it would seem as though any effect from $D_1^+$ and $B^-$ is removed, and the back reaction from $Q^-$ to P-680$^+$ is slow with kinetics similar to those observed in the cyanobacterial and green alga PS II preparations.

Thus a very slow back reaction with a life-time of about 600–1000 $\mu$s seems to occur from the state $[D_1||P680^+ \dots Q^-B]$.

The forward rate of electron transfer from $Q^-$

to B has been reported to occur with a half-time of about 150 $\mu$s [35]. Thus, it would seem that the state [P-680$^+$ ... QB$^-$] should be formed before the slower back reaction from Q$^-$ to P-680$^+$ occurs, as is the case in the reaction centres of photosynthetic bacteria [36]. The experiments with DCMU-inhibited fractions suggest that the slow 800 $\mu$s phase in P-680$^+$ reduction is not a back reaction from B$^-$, but rather Q$^-$. Thus, we find it difficult to explain the presence of the 800 $\mu$s phase in the absence of DCMU (when B$^-$ to Q electron transfer should occur). We would suggest that either (a) the electron can return from B$^-$ to Q in much less than 800 $\mu$s or (b) that when P-680$^+$ is oxidised, the electron transfer from Q$^-$ to B is inhibited.

Further details are required in order to decide whether it is the presence of an electron on B or the presence of a positive charge on $D_1$ which results in a faster back-reaction from Q$^-$ to P-680$^+$ in higher plant PS II fractions.

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