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PHOTOSYNTHETIC OXYGEN EVOLUTION FROM HYDROGEN PEROXIDE

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Summary

A study was made of the interactions of flash-illuminated chloroplasts with hydrogen peroxide. We conclude:

1. The oxygen precursor system can be reduced beyond the S_0 state to an S_{-1} state, which can be oxidized to S_0 by a single flash.
2. In the dark, a two-electron donation by H_2O_2 takes place which reduces S_2 to S_0 and S_1 to S_{-1}.
3. At the same time, two-electron oxidations by H_2O_2 re-form S_2 from S_0 and S_1 from S_{-1}.
4. The catalase-like activity due to this cyclic oxidation and reduction of the S enzyme is higher with the $S_2 \rightleftharpoons S_0$ couple than with the $S_1 \rightleftharpoons S_{-1}$ couple.

Another process, however, is responsible for most of the O_2 evolution from H_2O_2 in the light. Our evidence indicates that this process: (1) is independent of the S states and insensitive to Tris washing, (2) turns over rapidly in high concentrations of peroxide, (3) yields 1 O_2 per electron passing through system II; (4) dismutates two H_2O_2 molecules, so that there is no net consumption of 'holes'.

Introduction

Photosynthetic water oxidation is known to require a cycling of the donor complex of Photosystem II between five different oxidation states, S_0 to S_4 [1,2]. Presumably, the oxidation of artificial electron donors occurs by a much simpler mechanism, in one-equivalent steps [3]. One exception may be the oxidation of hydrogen peroxide [4—6]. There are some indications that this process may involve a cycling between three of the five S states [4]. Considering also that H_2O_2 could very well be chemically related to oxidation products intermediate between water and oxygen, we felt that a study of its interactions with Photosystem II might be of particular interest.

This paper reports attempts to further elucidate the light-induced oxygen evolution from hydrogen peroxide. We studied conditions in which the oxidations of H_2O_2 and H_2O compete, as well as those in which H_2O oxidation was absent due to extraction of manganese by washing with Tris [7] or hydroxylamine [8].

Materials and Methods

Spinach chloroplasts were isolated as described in ref. 9. For some experiments Tris-washed chloroplasts were used, prepared by incubating a concentrated chloroplast suspension (approx. 3 mM chlorophyll) during 15 min at 0°C in 0.6 M Tris · HCl (pH 10), followed by a 2000-fold dilution in standard medium (see below).

Chloroplast samples used in the polarograph were deposited as a thin layer on a Millipore filter (25 mm diameter) mounted in a plastic plate (~3 × 7 × 0.3 cm) which served as a sample holder. To prepare a sample, we used the slight suction of an aspirator to filter a 1 ml sample of a chloroplast suspension (~2 μg chlorophyll/ml) through a circular area (1 cm diameter) of the filter (0.45 μm bactoflex, Arthur Thomas Co.). The sample holders could be placed in (darkened) beakers containing immersion liquids, transferred to or dipped into another bath, etc., until they were finally placed under the polarograph electrode. Usually a series of 6—12 samples was prepared simultaneously. The samples were dark adapted for about 10 min at room temperature and then stored in the dark at 0°C until used. The standard medium contained 0.4 M sucrose/10 mM NaCl, 25 mM Tricine/NaOH (pH 7.8).

For oxygen measurements we used a Clark-type electrode consisting of a 5 mm diameter platinum button surrounded by a silver reference electrode. After being wetted with KCl solution, the electrode was covered by a silicon rubber membrane which was stretched and drawn tightly over the metal to improve the response time. The platinum was polarized at —0.6 V vs. the silver. The delay between a flash and the maximum height of the oxygen signal, when due to H_2O oxidation, was 0.1—0.2 s. Flashes were produced by E.G. & G FX 101 Xe-flash tubes (1100 V, 2 μF) and filtered by a yellow (Corning 3-68) cutoff filter. The measurements were performed at room temperature.

Results and Discussion

In their study of oxygen evolution from H_2O_2 by isolated chloroplasts Takahama et al. [6] showed that this process was only partly (50—70%) sensitive to the photosystem II inhibitor 3-(3,4-dichlorophenyl)-dimethylurea (DCMU). This result, which we confirmed, suggests that at least part of the H_2O_2 consumption activity might be due to photosystem I. We therefore compared the effect of beams of red and far-red light. These have strongly dissimilar relative efficiencies for system II reactions, like O_2 evolution from H_2O (Fig. 1A), and system I reactions, like O_2 uptake in the presence of methyl viologen and a system I electron donor (Fig. 1B) (e.g. ref. 10). For O_2 evolution from H_2O and H_2O_2, however, the relative efficiencies of the beams were approximately equal (Fig. 1A and C). We therefore conclude that direct donation of H_2O_2 to Photo-

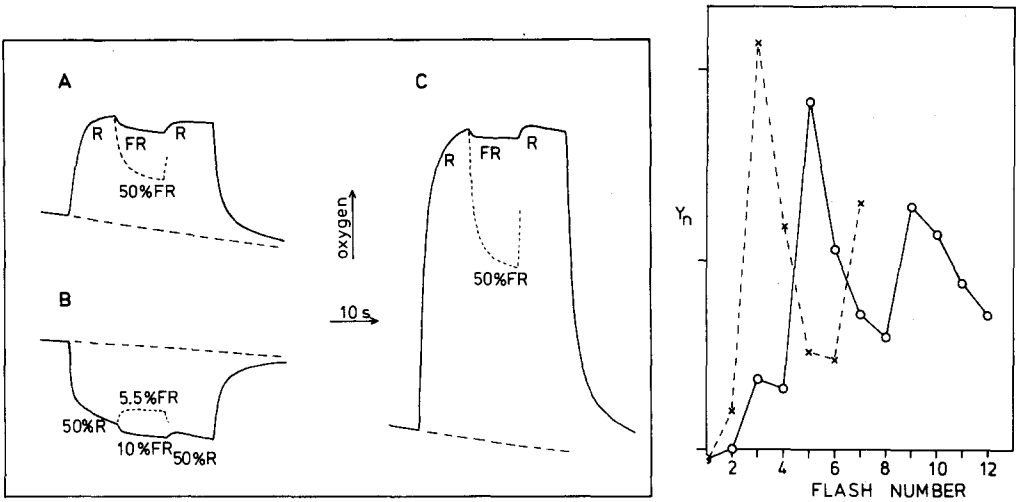


Fig. 1. Experimental traces of oxygen evolution or uptake in continuous red or far-red light. Dark-adapted samples were illuminated by a sequence of 10 s 640 nm light (R), 10 s 730 nm light (FR), 10 s 640 nm light (R). The amplitude of the signal is roughly proportional to the steady-state rate of oxygen evolution (or uptake), and evidenced by the half-signal obtained when 50% light intensity is used. The 100% intensities of 640 nm and 730 nm light are chosen to give approximately equal system II activity, on the order of 1 quantum absorbed per system II reaction center per s. (A) Oxygen evolution by oxidation of water in the presence of 0.5 mM ferricyanide. (B) Oxygen uptake by system I. Before the measurement, the chloroplasts were incubated in 1 mM hydroxylamine for 1 min, followed by a washing. Such treated chloroplasts showed zero activity under the conditions of the experiment of A. Additions to the standard medium: 5 mM ascorbate/1 mM diaminodurene/0.1 mM methyl viologen/catalase. (C) Oxygen evolution by oxidation of H_2O_2. Chloroplasts pretreated with hydroxylamine (see B). Additions: 0.5 mM ferricyanide/0.5 mM NaN_3/0.03% H_2O_2.

Fig. 2. Oxygen yields induced by a flash sequence (1 s spacing) in dark-adapted chloroplasts pretreated with H_2O_2 at pH 8.8 (○———○). Incubation for 1 min in standard medium at pH 8.8 containing 0.3% H_2O_2; then transfer to H_2O_2-free standard medium at pH 7.8 to which was added catalase, 0,1 mM ferricyanide; measurement after 3 min. x, oxygen yields of a flash sequence 5 min after the first series (20 flashes).

system I, if it occurs at all, does not play a significant role compared to oxidation by Photosystem II. The relative amplitude of the signals obtained in Figs. 1A and 1C will be discussed later in this paper. Elsewhere we will publish other evidence not involving H_2O_2, which indicates that, under some conditions, appreciable electron flow through system II is possible in the presence of DCMU.

In flash series experiments, the oxygen-yield patterns were significantly changed only by H_2O_2 concentrations at or above 0.001%. The effects included the evolution of (some) oxygen even at the first flash after dark adaptation. To observe the effects of such low concentrations of H_2O_2 it proved necessary to add a catalase inhibitor, such as sodium azide. Otherwise the H_2O_2 is evidently consumed by endogenous catalase: a few minutes after equilibration with H_2O_2, a single flash no longer produces oxygen.

Our measuring technique could only partly resolve the different phases of the oxygen evolution occurring when both H_2O and H_2O_2 contribute. To simplify matters, we will therefore present only the extreme cases: i.e. conditions in which all oxygen produced comes either from H_2O_2 or from H_2O. In the

latter experiments, pretreatments with low H_2O_2 concentration were made in the absence of a catalase inhibitor. Pretreatments with high concentrations were followed by exposure of the sample to a large volume of catalase-containing medium.

Absence of O_2 evolution after the first flash was used as a criterion for adequate removal of H_2O_2 (<0.001%, see above).

Reduction of S_1 to S_{-1} in the dark, the S_{-1} precursor state

A remarkable result obtained by the second procedure, pretreatment with H_2O_2 followed by its removal, is shown in Fig. 2. Chloroplasts were incubated for 1 min in the dark with 0.3% H_2O_2, pH 8.8, the hydrogen peroxide was then removed, and the pH returned to 7.8. 5 min after this treatment, the flash yield pattern was measured (open circles in Fig. 2). For the majority of the centers, a distinct two-step delay in oxygen production can be observed: five instead of three flashes are needed to produce oxygen from the initial state. In a second series of flashes, given 5 min after the first series, a normal pattern is again obtained (crosses in Fig. 2).

This effect of H_2O_2 strongly resembles that of preincubation with a low concentration of NH_2OH, as described by Bennoun and Bouges-Bocquet [11,12]. The latter effect was interpreted in terms of an irreversible binding of the artificial electron donor. Presumably, the initial S state still is S_1, but three flashes instead of one are now required to go from S_1 to S_2, the first two flashes being needed to oxidize the bound NH_2OH molecules. A similar explanation cannot apply in the case of H_2O_2. Oxidation of H_2O_2 in the first two flashes would have produced oxygen, which would have been detected in our measurement. Since it was not, we must conclude that the electron donation by H_2O_2 did not occur after the flashes, but had already occurred previously in the dark: S_1 had been reduced by a two-electron transfer. Apparently (in addition to the five states $S_0 \rightarrow S_4$), the oxygen system can occur in yet another (sixth) state. This state is quite stable (at least minutes) in dark; we denote it 'S_{-1}'.

The high pH used during incubation appears to be essential for the large effect shown in Fig. 2. At lower pH, incubation with H_2O_2 leads to qualitatively similar results, but the conversion to S_{-1} is less complete. Fig. 3A shows the effect of preincubation in 0.3% H_2O_2 at pH 7. Again, the occurrence of a two-step delay is evident, but now involves only a minority of the centers. The plusses in Fig. 3A show the flash yield pattern with control samples not subjected to H_2O_2 pretreatment. The data are not normalized: one observes that, as a secondary effect, the H_2O_2 pretreatment causes the inhibition of some of the centers (30% inactivation was typical).

The incomplete reduction to S_{-1} at near neutral pH does not seem to be due to insufficient incubation time. Fig. 3B shows the effect of H_2O_2 as a function of the incubation time. Incubation for 1 min in 0.3% H_2O_2 suffices to obtain the maximal effect. The incompleteness of the conversion therefore implies the attainment of a 'steady state'; i.e. not only the S_1 to S_{-1} reaction occurs, but also the back oxidation S_{-1} to S_1. Only at high pH do the reactions favor the S_{-1} state. If correct, this interpretation predicts that pretreatment with H_2O_2 at pH 8.8 should be 'forgotten' when it is followed by an incubation with high H_2O_2 at lower pH. This has indeed been observed (not shown).

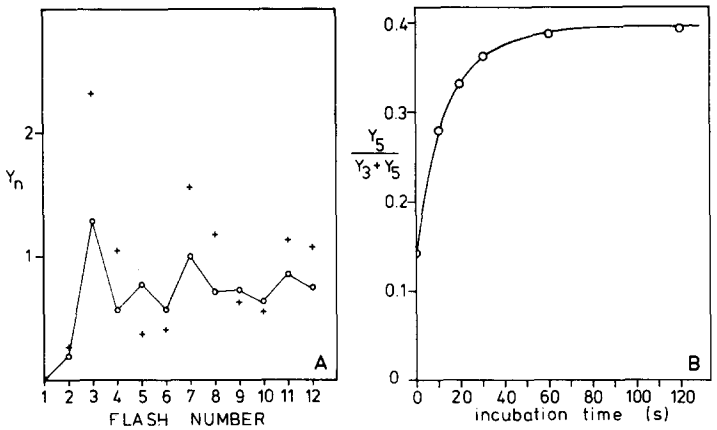


Fig. 3. (A) Oxygen yields induced by a flash sequence (1 s spacing) in dark-adapted chloroplasts pretreated with H_2O_2 at pH 7 (○ - - - - - - ○). Pretreatment: 1 min in standard medium at pH 7.0 containing 0.3% H_2O_2, followed by 4 min in H_2O_2-free standard medium containing catalase, 0.1 mM ferricyanide, and 0.1 mM ferrocyanide. +, no H_2O_2 pretreatment. (B) Incubation time dependence of the change in oxygen-yield pattern induced by preincubation of chloroplasts with 0.3% H_2O_2 at pH 7. Conditions as in A, except for pretreatment time.

When chloroplasts are illuminated with a single flash after (or just before the end of) the exposure to a high H_2O_2 concentration, followed by a wash in dark, a subsequent flash series shows a one-step delay in the oxygen yield pattern instead of the two-step delay (Y_4 being the maximum flash yield in a series). This result can be readily explained as being due to a flash-induced transformation of S_{-1} into S_0.

The reduction of $S_1 \rightarrow S_{-1}$ coupled to the oxidation of H_2O_2 is interesting from a viewpoint of energetics. The two-electron oxidation of peroxide has a midpoint potential of (at pH 7) 280 mV; therefore, the potential of the S_1/S_{-1} couple must be higher than this value.

At high pH, H_2O_2 is more strongly reducing, which might accelerate the $S_1 \rightarrow S_{-1}$ reaction. It is also less oxidising, which might decrease the rate of the reconversion $S_{-1} \rightarrow S_1$. The increase in the ratio S_{-1}/S_1 at high pH may have been due to either one of these effects.

Reduction $S_2 \rightarrow S_0$ in dark

Fig. 4 (open circles) shows that, at pH 7.8, an exposure to 0.03% H_2O_2, which is consumed within a few minutes by endogenous catalase, induces only a small number of S_{-1} states. The flash yield pattern observed with such a sample differs only slightly from that obtained with untreated, control samples (plusses in Fig. 4). Evidently, becuase of the low concentration and brief exposure, the reaction $S_1 \rightarrow S_{-1}$ did not proceed appreciably. However, when a single flash is given, 10 s after the addition of H_2O_2, the pattern (measured 5 min later when all peroxide is gone) is more strongly affected. A high ratio Y_4/Y_3 is seen, indicating that an appreciable formation of S_0 has taken place (dots in Fig. 4). Note that, in this experiment, the effect of the preflash is opposite to that observed in the absence of H_2O_2, normally a single flash, followed by dark deactivation leads to high value of Y_3, i.e. a decrease rather than

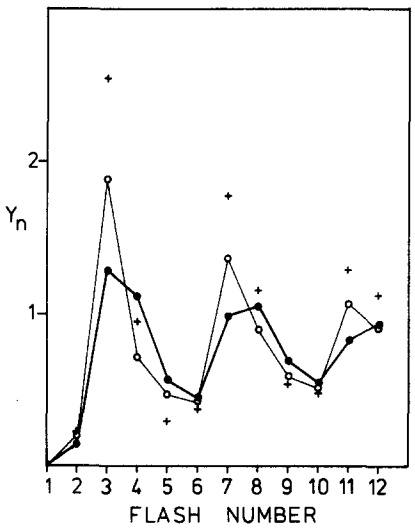


Fig. 4. Oxygen yields induced by a flash sequence (1 s spacing) in dark-adapted chloroplasts pretreated with 0.03% H_2O_2 at pH 7.8. Pretreatment: exposure to standard medium to which was added: 0.03% H_2O_2/0.1 mM ferricyanide/0.1 mM ferrocyanide. Measurement 5 min later; the H_2O_2 concentration had at that time dropped to below 0.001%, presumably due to endogenous catalase. ○———○, no preflash; ●———●, 1 preflash was given approx. 10 s after the exposure of the chloroplasts to the H_2O_2 solution.

an increase of S_0 [1,2]. This result indicates that in the presence of peroxide, S_2 made from S_1 by the flash is reduced to S_0 in the dark. Evidently, this two-equivalent reduction $S_2 \rightarrow S_0$ occurs with a higher rate than the reduction $S_1 \rightarrow S_{-1}$. This interpretation is supported by measurements of oxygen production in the presence of H_2O_2, as described below.

$S_0 \rightarrow S_2$ in dark, catalase-like activity associated with the S states

Oxygen evolution from H_2O_2 without concomitant oxidation of water can be observed under a number of conditions. Some examples are given in Fig. 5, which shows the actual electrode responses. For comparison, Fig. 5a shows the response during oxygen evolution from water, effected by three flashes in the absence of H_2O_2. In this case, the signal reaches a maximum in about 0.15 s and then decays as oxygen is consumed and diffuses away from the electrode; the area under the trace represents approx. 0.6 O_2 per reaction center [1]. Fig. 5b shows the O_2 evolution after the first flash given to a dark-adapted sample containing 0.3% H_2O_2 and 10^{-3} M azide. The signal maximum is displaced to approx. 0.3 s, and, in addition, the decay is slower. Because of the persistent slow O_2 evolution, the area under the trace was difficult to evaluate (see later). Using the curve of Fig. 5a for calibration, we computed that the area under the trace up to 10 s after the flash represented about 1 O_2 per center.

At this peroxide concentration, washing with hydroxylamine had little effect upon the yield of the (first) flash.

Fig. 5c shows O_2 evolution after the first flash, as in Fig. 5b, but now a higher concentration (0.3%) of peroxide was present. In this case, the amount of O_2 evolved was many times the number of reaction centers (the area under the trace up to 10 s was approx. 4 O_2 per center). The slow phase of O_2 evolution

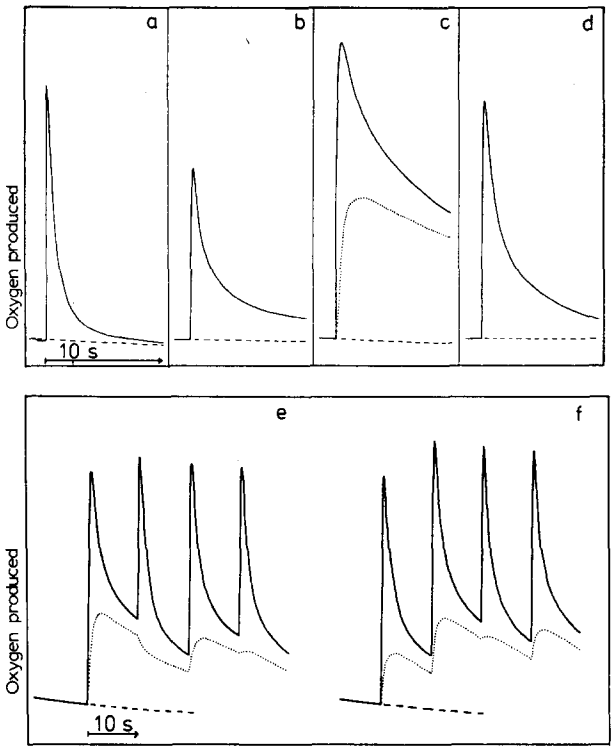


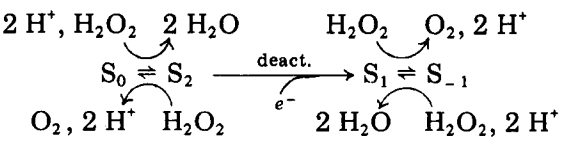
Fig. 5. Experimental traces for flash-induced oxygen evolution in the absence and presence of H_2O_2. Pretreatment: 3 min exposure to standard medium to which was added 0.1 mM ferricyanide, 0.1 mM ferrocyanide, 0.1 mM NaN_3, and various concentrations of H_2O_2. (a) H_2O_2 absent: three flashes given (dark times between flashes 1 s and 10 ms). (b) Single flash given in the presence of 0.03% H_2O_2. (c) Single flash given in the presence of 0.3% H_2O_2; dotted line, slow component, difference between traces c and d. (d) Like c, but the sample had first been incubated for 1 min in medium containing 1 mM NH_2OH, then washed before being transferred to the medium containing H_2O_2. All pretreatments were performed in the dark. Parallel samples used for a–d. (e) Four flashes were given at intervals of 10 s in the presence of 0.3% H_2O_2. (f) Like e, except that five flashes were given, and the interval between the first two flashes was 10 ms. The dotted lines in e and f show the assumed behavior of the slow component (cf. c). Parallel samples used for e and f.

is large and extended. In this case, washing with Tris or hydroxylamine has a pronounced effect, as is shown in Fig. 5d. Most of the slow evolution disappears and the shape of the remaining signal is very similar to that in Fig. 5b. Peak height and the ($\leqslant$10 s) area are about 30% higher, an aspect which will be discussed later (see Fig. 6). We conclude that, with fresh chloroplasts and high peroxide concentrations, the flash triggers the slow oxidation of a considerable amount of peroxide. A rough estimate of the electrode response due to this process is the difference between traces 5c and d (dotted trace in 5c).

We can now attempt to interprete this process. We had previously concluded that both the reduction of S_1 to S_{-1} and the oxidation of S_{-1} to S_1 proceed continuously in the dark in the presence of H_2O_2. This implies that, in the dark, system II sustains a catalase-like activity: in a cycle from S_1 via S_{-1} back to S_1, two molecules of H_2O_2 will be dismutated into two water molecules and one oxygen. This process is quite slow (according to Fig. 2b, the step $S_1 \rightarrow S_{-1}$ takes approx. 20 s in 0.3% H_2O_2) and should give only a small, barely detect-

able, increase of the "background" oxygen signal. Furthermore, we concluded that the S_2 to S_0 reaction proceeds more rapidly than the S_1 to S_{-1} transformation. To explain the large oxygen evolution after one flash as represented by the dotted trace in Fig. 5c, we assume that the S_0 to S_2 oxidation can also be induced by H_2O_2 similar to the S_{-1} to S_1 oxidation. In other words, the flash transforms system II from a low-activity catalase, cycling between S_{-1} and S_1, to a higher-activity catalase, cycling between S_0 and S_2. (At 0.3% H_2O_2, the respective rates are on the order of <1 O_2 per 20 s and approx. 1 O_2 per 3 s). Figs. 5e and f provide additional support for this hypothesis. In a flash series, given in the presence of high $[H_2O_2]$, the amplitude of the slow phase is larger after an odd than after an even number of flashes. Presumably, consecutive flashes shift the system back and forth between the S_1, S_{-1} and S_2, S_0 couples. A distortion of the binary oscillation (e.g. in Fig. 5e the slow phase is slightly increased by the fourth flash) is due to deactivation between the flashes. The oscillation of the slow phase becomes more obvious when Figs. 5e and 5f are compared. In Fig. 5f, the first flash was replaced by a closely spaced (10 ms) flash pair, which yielded considerably less O_2 than a single flash; the third flash, given 10 s after the flash pair again triggered a high production, as it did in the experiment of Fig. 5e.

Scheme 1 illustrates the proposed sequence of events:



Scheme 1.

It includes the deactivation reaction $S_2 \rightarrow S_1$ to account for the decay of the $S_0 \rightleftharpoons S_2$ catalase activity in the course of 20—40 s. In the experiments of Figs. 5e and f, the first two flashes show no deficit and no periodicity of four is detectable. Apparently, O_2 evolution from water is suppressed. In the context of the above scheme, some S_2 should be present at all times and transformed by a flash to S_3. It seems plausible that S_3 is unstable in the presence of H_2O_2 and is reduced to S_1.

Photochemical H_2O_2 dismutation unrelated to the S states

With long flash intervals (e.g. 10 s) water oxidation is not observed even with the low H_2O_2 concentrations (0.03%) used in the experiment of Fig. 5b. (With spacings $\leqslant 1$ s, a period of four oscillation can be recognized). At such low H_2O_2 concentrations also the slow catalase-like effects of Scheme 1 are inconspicuous; following each flash one observes a relatively rapid spike of O_2 evolution (Fig. 5b) and no oscillation is seen. Since dark relaxation can be rather rapid, the spike can be induced many times per second and this additional, "rapid" process is by far the dominant path of H_2O_2 decomposition in any but the weakest light intensities. Whereas treatment with Tris or hydroxylamine annihilates the slow processes of Scheme 1, it hardly affects the rapid, flash-induced spikes (compare Fig. 5b and d). We evidently are dealing with a process that is independent of the S states, does not require the O_2-generating sys-

tem (the "large" Mn pool of O_2 evolution [13]), and bypasses it in intact chloroplasts. Fig. 6 shows the effect of H_2O_2 concentration upon the O_2 flash yield measured with Tris-washed chloroplasts, so that this process was the only source of O_2 evolved from H_2O_2. Beyond 0.01% the shape of the flash yield trace is not influenced by H_2O_2; the magnitude of the yield saturates at about 0.05% H_2O_2.

We have no ready explanation for the biphasic nature of the curve. Other evidence also indicates that we are dealing with complicated events. For instance: compared to Fig. 5a, the traces in Figs. 5b and d show a slow component, somewhat resembling the much larger slow component in Fig. 5c. We suspect that this slow component occurs only after the first flash following a long dark period. This suspicion rests on the following argument:

In Fig. 1 trace A, the steady-state rate of O_2 evolution from water represents about 0.25 O_2 per hit system II reaction center. In trace C, the rate of O_2 evolution from peroxide is approx. three times higher and thus represents 0.75 O_2 per hit per center. Had a saturating concentration of H_2O_2 been used in this experiment, this number would have been 30% higher (Fig. 6) or approx. 1 O_2 per hit per center.

In a similar fashion the area under the trace in Fig. 5a (0.6 O_2 per trap) can be used to estimate the amounts of O_2 evolved in the other experiments of Fig. 5.

In Fig. 5d, the area under the trace up to 10 s is 1.4 O_2; the total area might be $\geqslant 2$ O_2 per flash. Possibly the fast and the slow phase each contribute 1 O_2/trap. Because, in an illumination of 1 hit per s as used in Fig. 1, the rate is only approx. 1 O_2 per s, we conclude that in this case, the slow phase does not contribute.

Close inspection of the traces of Figs. 5a and d shows that the rise time of

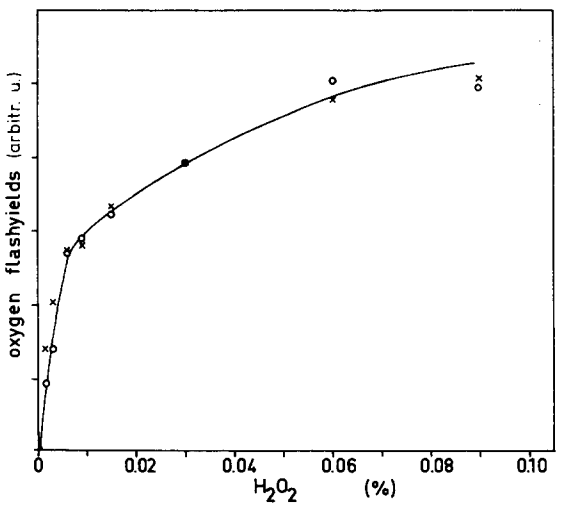


Fig. 6. Oxygen flash yields of Tris-washed chloroplasts as a function of H_2O_2 concentration. Additions: 0.1 mM ferricyanide, 0.1 mM ferrocyanide, 0.5 mM NaN_3, various concentrations of H_2O_2. A fresh sample, dark-adapted and exposed to H_2O_2 for 3 min, was used for each point. Illumination by a single flash. ○, peak-height; x, area below the trace up to 5 s.

signal 5d (which should be determined almost entirely by the rapid phase of peroxide decomposition) is somewhat slower than the rise of signal 5a.

In experiment 5a the O_2 evolution occurs within approx. 1 ms after the flash [14] so that the time course of the observed signal is determined entirely by the time response of the membrane covered electrode. The slower rise time of the trace reflecting O_2 formation from peroxide implies that this formation occurs in a time interval which is comparable to the response time of the electrode (approx. 0.1 s).

Such times are indeed observed in the experiments of Fig. 7, which shows the O_2 yield of a flash pair as a function of the time interval separating the two flashes. Neglecting for a moment the events occurring at very brief spacings, we note that the ascending slope of the four curves is proportional to the H_2O_2 concentration and shows no sign of saturation at the highest value used (0.12% where the relaxation is half at less than 10 ms).

Remarkably, when the ascending slopes are extended to zero time, it appears that they originate at a common point, which is only approx. 40% of the yield of a single flash. Accordingly, with short flash intervals, the yield of a pair is below, rather than above, that of a single flash. The rate of the initial decline increases with $[H_2O_2]$ until it becomes constant at concentrations above 0.05%.

We will not venture a detailed interpretation of these complicated phenomena. Apparently, there are two phases in the oxidation of H_2O_2 by extracted chloroplasts: a center hit in an intermediate state after completion of the first phase, but before completion of the second, is no longer able to complete the second phase. Since the kinetics of both phases are dependent on the H_2O_2 concentration, it seems that each center reacts with more than one molecule of H_2O_2. This suggests that we may be dealing with a catalyzed dismutation (one

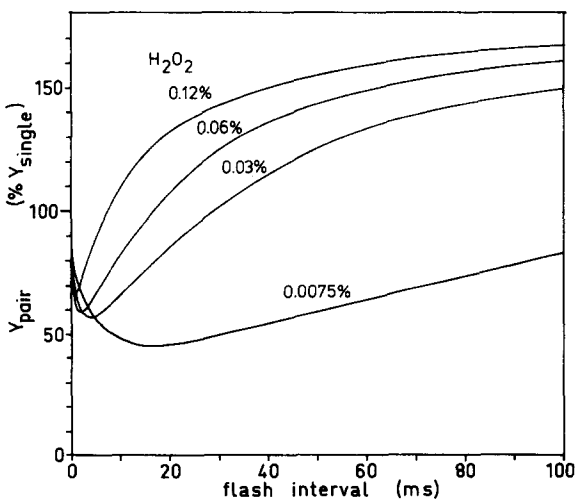


Fig. 7. Oxygen yield of a flash pair observed with Tris-washed chloroplasts as a function of flash interval at various H_2O_2 concentrations. Additions: 1 mM ferricyanide, 1 mM NaN_3, various concentrations of H_2O_2. Each curve was measured with a single sample which was alternatively illuminated by a flash and a flash pair at 10-s intervals. At each H_2O_2 concentration, the yields of the flash pairs were normalized to those of the single flashes (taken as 100%).

molecule of H_2O_2 accepting and another molecule of H_2O_2 donating an electron pair) rather than with a net electron donation of H_2O_2 to the photosystem. Although some net electron flow from hydrogen peroxide has been observed in extracted chloroplasts [5], we believe that the main path is dismutation. An additional strong argument for this conclusion rests on the flash yield of one (rather than 1/2) O_2 molecule per reaction center, as computed earlier in this section.

Acknowledgements

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