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OXYGEN-EVOLUTION PATTERNS FROM SPINACH PHOTOSYSTEM II PREPARATIONS

MICHAEL SEIBERT [a] and JEAN LAVOREL [b]

[a] *Solar Energy Research Institute* *, *Golden, CO 80401 (U.S.A.) and* [b] *Laboratoire de Photosynthèse, CNRS, 91190 Gif-sur-Yvette (France)*

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Patterns of O_2 evolution resulting from sequences of short flashes are reported for Photosystem (PS) II preparations isolated from spinach and containing an active, O_2-evolving system. The results can be interpreted in terms of the S-state model developed to explain the process of photosynthetic water splitting in chloroplasts and algae. The PS II samples display damped, oscillating patterns of O_2 evolution with a period of four flashes. Unlike chloroplasts, the flash yields of the preparations decay with increasing flash number due to the limited plastoquinone acceptor pool on the reducing side of PS II. The optimal pH for O_2 evolution in this system (pH 5.5–6.5) is more acidic than in chloroplasts (pH 6.5–8.0). The O_2-evolution, inactivation half-time of dark-adapted preparations was 91 min (on the rate electrode) at room temperature. Dark-inactivation half-times of 14 h were observed if the samples were aged off the electrode at room temperature. Under our conditions (experimental conditions can influence flash-sequence results), deactivation of S_3 was first order with a half-time of 105 s while that of S_2 was biphasic. The half-times for the first-order rapid phase were 17 s (one preflash) and 23 s (two preflashes). The longer S_2 phase deactivated very slowly (the minimum half-time observed was 265 s). These results indicate that deactivation from $S_3 \rightarrow S_2 \rightarrow S_1$, thought to be the dominant pathway in chloroplasts, is not the case for PS II preparations. Finally, it was demonstrated that the ratio of S_1 to S_0 can be set by previously developed techniques, that S_0 is formed mostly from activated S_3 (S_4), and that both S_0 and S_1 are stable in the dark.

Introduction

Algal and green plant photosynthesis employs light reactions in two sequential photosystems to provide energy for the organism and to fix CO_2. Stored reductant in the form of carbohydrate is used by the organism to drive cellular metabolism and ultimately to generate biomass. However, in addition to light and CO_2, protons and electrons also are required for carbon reduction. The plant obtains both by splitting water in a process that produces O_2 as a waste product. Consequently, photosynthetic water splitting is an important fundamental process for plant survival and one that has been actively studied over the past 15 years. Recent reviews by Govindjee [1] and Velthuys [2] are available.

The process of water splitting can be probed by monitoring oxygen evolution, fluorescence and luminescence resulting from very short, single-turnover flashes of light; and extensive studies

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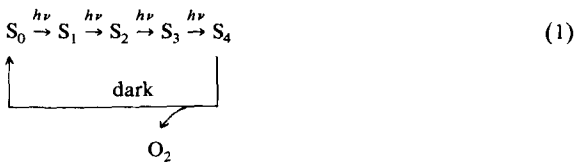
Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid; PS, photosystem; S_n, oxidation level of the oxygen-enzyme system with $n = 0, 1, 2, 3$, or 4; Y_n, oxygen flash yield after flash n.

have been recorded with algae and chloroplasts [3,4]. Over the past 4 years, but particularly within the last $1\frac{1}{2}$ years, several groups [5–8] have reported procedures yielding detergent-fractionated PS II preparations that contain an active water-splitting, manganese-containing enzyme. This new system permits investigation of water oxidation on a preparation that contains the heretofore minimum structural material required for function. Consequently, it provides a simpler complex for assessing the validity of the 'S-state' model first developed as in Eqn. 1 to explain O_2 evolution in chloroplasts [9]:



(1)

The model requires that the Mn-enzyme center exists in any one of five different oxidation S-states. Each photon absorbed by a PS II reaction center in the alga or chloroplast forms a strong oxidizing equivalent that can advance an S-state from S_n to S_{n+1} ($n = 0–3$) by extracting an electron from the enzyme (protons also are involved but are ignored here). In addition, S_0 and S_1 are stable in the dark, S_2 and S_3 can deactivate mostly to S_1 in the dark (though the exact deactivation scheme is still debated), and S_4 is transformed to S_0 in the dark, releasing an O_2 molecule in the process.

In this paper, we report on patterns of oxygen evolution from O_2-evolving PS II preparations elicited by sequences of short flashes. The results are in general consistent with the S-state model, but several anomalies have been observed. Anomalous O_2-production patterns have been reported previously in O_2-evolving PS II preparations [10].

Materials and Methods

Biological samples. chloroplasts were prepared by the method of Robinson and Yocum [11] from market spinach purchased during the late spring and summer months. O_2-evolving PS II samples were prepared from spinach using sequential treatments of digitonin and Triton X-100 (both from Sigma Chemical Co., St. Louis) according to the procedure of Yamamoto et al. [12] except that Mes was substituted for $Na_4P_2O_7$ (Nishimura, M., personal communication). This modification did not affect the results of the flash studies, but gave preparations with higher O_2-evolution rates on a chlorophyll basis and fewer SDS-polyacrylamide gel electrophoresis [13] bands. PS II samples were suspended in 10 mM Mes (pH 6.5), 4 mM $MgCl_2$ and 0.33 M sorbitol at 4°C and used fresh or stored at −80°C.

Assays. Chlorophyll analyses were performed according to the method of Arnon [14]. Hill reaction (PS II) activity was measured at 25°C under saturating tungsten light with a water filter using a Clark-type O_2 electrode (YSI Model 5331, Yellow Springs, OH). The reaction medium included 1 mM 2,6-dimethyl-*p*-benzoquinone; 2 mM potassium ferricyanide; 3 mM NH_4Cl; and 10 mM Mes (pH 6.5), 15 mM NaCl, 5 mM $MgCl_2$ and 0.4 M sucrose buffer. O_2 uptake or PS I activity was assayed [15] under the same conditions using 75 μM 2,6-dichlorophenolindophenol, 2.5 mM L-ascorbic acid (sodium salt), 10 μM DCMU, 3 mM NH_4Cl, 30 μg methyl viologen/ml, 2 μl bovine liver catalase solution (No. C-100, Sigma Chemical Co.) and the same pH 6.5 Mes/NaCl/$MgCl_2$/sucrose buffer. O_2-evolution rates for the PS II preparations ranged between 380 and 440 μmol O_2/mg Chl per h and O_2-uptake rates varied from 5 to 13 μmol O_2/mg Chl per h.

Rate electrode. O_2 yields, elicited by trains of saturating 3-μs xenon flashes utilizing a modified PRA Model 6100B (London, Ontario) flash source, were examined at 23°C with a Joliot-type O_2-rate electrode, modified from the model described in Ref. 16. No added electron acceptors were used in the samples or flow buffer.

Results

Flash-yield sequences

Fig. 1 compares flash-yield sequences of dark-adapted O_2-evolving PS II preparations with those of chloroplasts. Note that up to flash No. 4, there is no significant difference in the results. The chloroplasts show damped oscillations with a period of four and reach a steady-state value of about half the yield of the third flash. These results are normal and have been demonstrated before [9,17]. The PS II samples also show damped

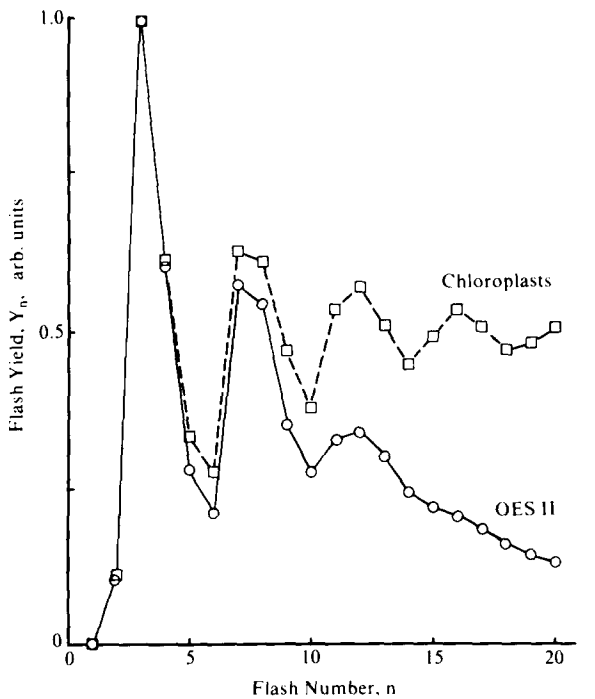


Fig. 1. A comparison of room temperature (23°C) chloroplast and O_2-evolving PS (OES) II O_2-flash-yield (Y_n) sequences. The samples were dark adapted for 10 min with no preillumination and then exposed to 20 flashes, each spaced 1.5 s apart. Chloroplasts, 1.50 mg Chl/ml; O_2-evolving PS II, 1.34 Chl/ml; rate-electrode flow buffer, 15 mM NaCl, 5 mM $MgCl_2$, 100 mM KCl and 20 mM Hepes (pH 7.5) for chloroplasts or 10 mM Mes (pH 6.5) for O_2-evolving PS II preparations. Results were normalized at Y_3.

oscillations, but instead of reaching a steady-state level, the oscillations appear superimposed on a function that decays towards the baseline. (The reader will recall that no terminal acceptor was added except that O_2 was present in the experimental system.) These results are similar to those we reported for O_2-evolving PS II preparations made by other techniques [10], but they differ in that anomalous O_2 evolution is not detected on the first flash.

Fig. 2 reports the pH dependence of Y_3 (the O_2 yield of the third flash) for both chloroplasts and O_2-evolving PS II. Note that the pH optimum range for the O_2-evolving PS II preparations is pH 5.5–6.5 and that the yield falls off rapidly at more alkaline pH values. On the other hand, the chloroplast yields are constant from pH 6.5 to 8.0 and fall off at more acidic pH values.

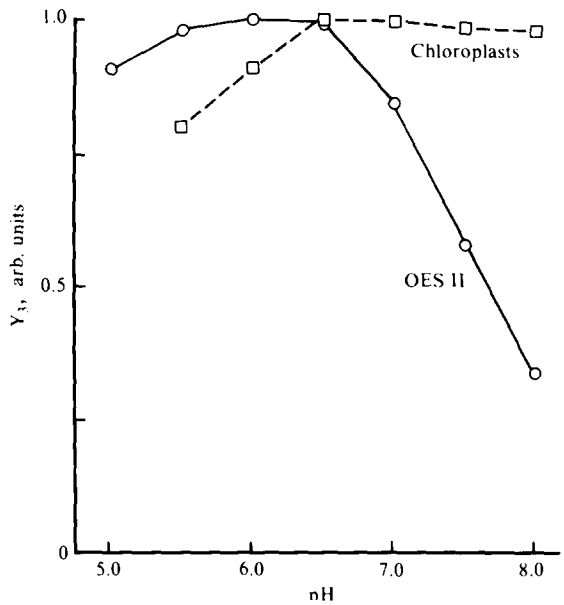


Fig. 2. The effect of pH on the oxygen yields (Y_3) for chloroplasts and O_2-evolving PS (OES) II samples. Conditions were the same as Fig. 1 except that the flow buffer contained 10 mM citrate for pH 5.0, 10 mM Mes (O_2-evolving PS II) or 10 mM $Na_4P_2O_7$ (chloroplasts) for pH 5.5–6.5, and 10 mM Hepes for pH 7.0–8.0. Chloroplasts, 1.33 mg Chl/ml; dark-adaptation time, 5 min. Results were normalized at pH 6.5, and a new sample was used for each data point.

Inactivation

Fig. 3 shows the inactivation (irreversible loss of activity) kinetics of chloroplasts and O_2-evolving PS II samples which were dark adapted on the rate electrode at room temperature. The decay of Y_3 is first order in both cases, and the half-times are 32 and 91 min, respectively. However, if one looks at the inactivation kinetics of Y_{19}, the situation is different. In the case of O_2-evolving PS II, the flash yield seems to increase for about the first 30–40 min and then decreases with about the same slope as that of Y_3. On the other hand, the decay of Y_{19} in chloroplasts is quite similar to that of Y_3.

Deactivation

The experiments depicted in Fig. 4A and B for O_2-evolving PS II preparations used protocols similar to those developed for algae and chloroplasts by previous investigators [3,4]. In Fig. 4A, dark-adapted samples were exposed to two preflashes, various dark times, and then four probe flashes.

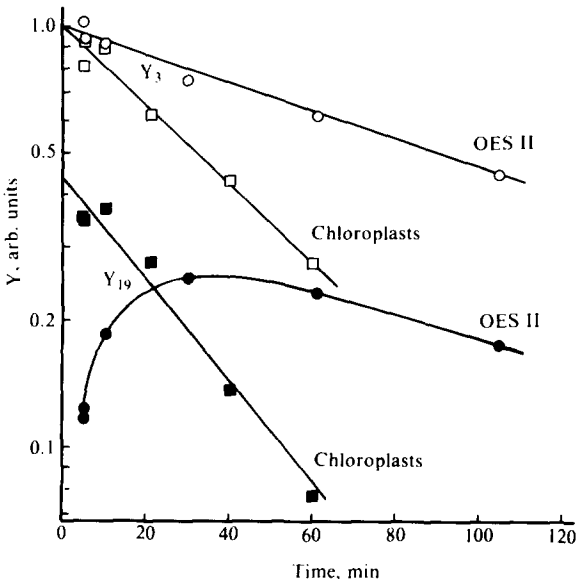


Fig. 3. Inactivation kinetics of chloroplasts and O_2-evolving PS (OES) II preparations. Conditions were the same as those of Fig. 1 except that Y_3 or Y_{19} was plotted against the dark-adaption times. Chloroplasts, 1.33 mg Chl/ml; O_2-evolving PS II, 1.39 mg Chl/ml. A new sample was used for each data point.

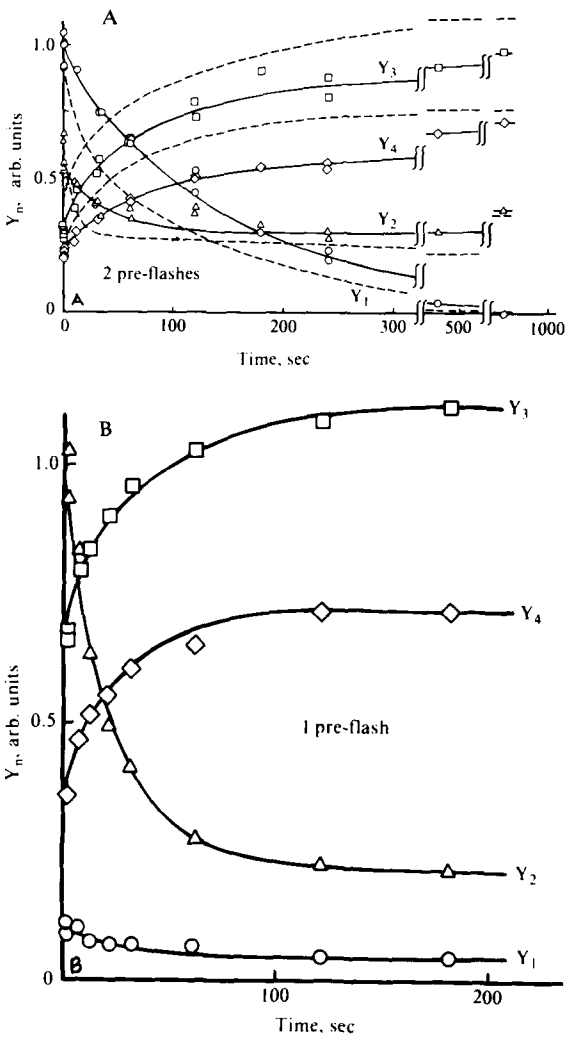


Fig. 4. (A) Deactivation of dark-adapted (5 min) O_2-evolving PS II samples following pre-illumination with two flashes. Oxygen yields for four probe flashes are plotted as a function of the dark time between the second preflash and the first probe flash, and a fresh sample was used for each dark time. Conditions were the same as those of Fig. 1 except the chlorophyll concentration was 1.08 mg/ml. The dashed curves represent samples exposed to 25 preflashes prior to the first 5 min dark-adaption period. Y_1 at time zero was set at 1.0, but no other normalization was made. (B) Same as A except that only one preflash was used. The data were plotted (not normalized) on the same scale as that in A.

O_2 evolution resulting from each of the probe flashes (Y_n, $n = 1-4$) was plotted as a function of the dark time between the second preflash and the first probe flash. The dashed curves represent the results when the preparations were exposed to 25 flashes prior to the dark-adaptation step, and they demonstrate previously noted observations that the kinetics can vary with conditions used to obtain the data [4]. The data in Fig. 4B were obtained under conditions similar to those of Fig. 4A except that only one preflash was used. In general, the results with the PS II preparations are similar to those with *Chlorella* (Fig. 2 of Ref. 3) and spinach chloroplasts (Figs. 6 and 7 of Ref. 4), but there are some obvious differences. For example, in the two preflash case, the decay of Y_2 appears to be clearly biphasic. The first phase decays faster than Y_1, and the second phase does not appear to decrease at all in this sample at times greater than 200 s. Another O_2-evolving PS II sample (data not presented) also showed biphasic decay but the second component deactivated with a long but measurable half-time (265 s). In the one-preflash case, Y_2 decay again appears to be biphasic, and the ratio of Y_4 to Y_3 at long times is greater than that observed for chloroplasts [3,4] though similar to that in *Chlorella* [3].

Fig. 5 plots the decay kinetics of Y_1 in Fig. 4A.

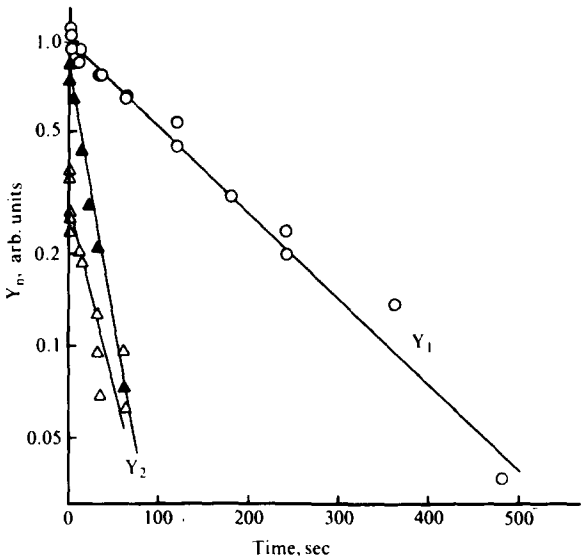


Fig. 5. Deactivation kinetics of S_3 (Y_1, Fig. 4A, ○) and S_2 (Y_2, Fig. 4B, ▲; Y_2, Fig. 4A, △). The conditions were the same as those in Fig. 4A and B, but the slow component of Y_2 was subtracted prior to plotting.

In this case the two preflashes convert any S_1 in the preparations to S_3 (see Eqn. 1), ignoring misses and double hits [18], and consequently the O_2 emitted as a result of the first probe flash (Y_1) is a measure of the amount of S_3 remaining. Deactivation of S_3 appears to be first order with a half-time of 105 s in contrast to results with algae [3,19] and chloroplasts [3,4] which were reported to be second order * with half-times in the range 6–30 s. The S_3 deactivation time we reported [10] previously for a different type of O_2-evolving PS II preparation was considerably longer (approx. 400 s). Also included in Fig. 5 are the decay kinetics of Y_2 for both the one- and two-preflash cases of Fig. 4B and A, respectively. In the first case, one can observe the deactivation of S_2 (as Y_2) with little interference from S_3 while in the second case interaction between S_2 (as Y_2) and S_3 (as Y_1) should be observable. The decay kinetics of the rapid phase seem to be first order, and the half-times for S_2 in the two cases are 17 and 23 s, respectively.

* Actually, replots of the published chloroplast data of both Joliot et al. (Fig. 3B of Ref. 3) and Forbush et al. (Fig. 7 of Ref. 4) appear as neither first nor second (assuming equal concentrations of two reactants) order but rather, more closely follow a log (time) decay curve.

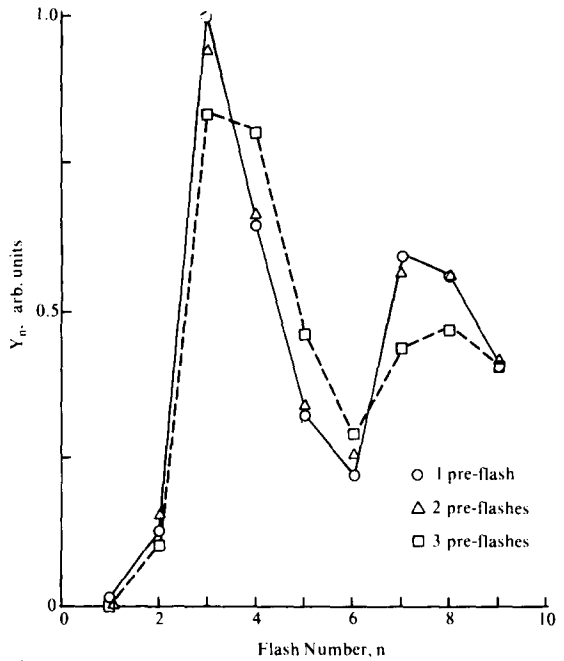


Fig. 6. O_2-flash yield sequences as a function of the number of preflashes in dark-adapted (5 min) O_2-evolving PS II preparations. The samples were exposed to a 10 min dark period between the last preflash and the first probe flash. Other conditions were the same as those in Fig. 1.

Setting the ratio of S_1 to S_0

Fig. 6 shows changes in the sequences of flash yields resulting from changing the initial ratio of S_1 to S_0. The ratio is controlled by exposing the O_2-evolving PS II preparations to one, two or three preflashes. In dark-adapted samples, one flash will result in the highest S_1-to-S_0 ratio (since any S_0 will be converted to S_1 and any higher S-states produced will deactivate to S_1) whereas three flashes will give a lower ratio (since S_1, the predominant state in dark-adapted samples, will be converted to S_0 as depicted in Eqn. 1). That changes in the ratio of S_1 to S_0 do in fact occur in O_2-evolving PS II preparations is demonstrated by changes in Y_4/Y_3 and Y_8/Y_7 ratios.

Discussion

O_2-yield patterns

The damped oscillations of the O_2-flash yields in chloroplasts reach a steady-state value (Y_{ss}) while those in O_2-evolving PS II preparations decay towards zero (Fig. 1). The chloroplast se-

quence reaches a nonzero Y_{ss} in spite of the fact that there is no terminal electron acceptor in the system. The likely explanation is that O_2 itself serves as an electron acceptor on the reducing side of PS I (Mehler reaction). This reaction is presumably slow and therefore is not recorded by our differentiating instrument which only senses fast O_2-concentration changes. In agreement with this explanation, we have observed that Y_{ss} decreases with increasing flash rate (data not shown; see also Ref. 20). Presumably, under such conditions the plastoquinone pool is reduced faster than it can be reoxidized by PS I and the Mehler reaction.

As discussed in a preliminary note [10], the decay of the oxygen yield in O_2-evolving PS II preparations is due to the limited amount of plastoquinone acceptor pool available to PS II and the fact that the pool is not reoxidized by PS I. When the pool is reduced, the primary photochemistry of PS II short-circuits (Ref. 21 and references therein), no strong oxidizing equivalents are generated, the water-splitting enzyme does not function and O_2 evolution stops. In contrast to our previous results [10], anomalous O_2 production on the first flash was not observed. To explain the discrepancy we note that the S_3 deactivation half-time measured for O_2-evolving PS II samples in this study was approx. 100 s (Fig. 5) compared to about 400 s observed before [10]. Therefore, complete deactivation of S_3 prior to an experiment was much faster in the present case, and the lack of complete S_3 deactivation might have led to the anomalous observation in the previous case (though special care was taken to minimize this possibility). The O_2-evolving PS II samples used in the current study were prepared by sequential dititonin and Triton extraction [12] whereas those used in the previous work were extracted with a higher concentration of Triton alone [8]. However, the method of preparation did not seem to be critical because new preparations of Triton samples revealed no anomalous O_2 on the first flash. Since the anomalous O_2 results we reported previously were observed with preparations made from market spinach grown in the winter (purchased locally in February and March) and since the current results are with spinach purchased in May to July, we believe that the source of the spinach may have a great influence on the S_3 deactivation rate and that this is the mostly likely explanation for the discrepancy. Aging of June spinach at 4°C prior to O_2-evolving PS II preparation did not result in the observation of O_2 on the first flash.

Inactivation

Fig. 3 shows that the half-time for the inactivation of the digitonin/Triton O_2-evolving PS II samples as indicated by the decay of Y_3 is 91 min at room temperature. For comparison, the inactivation half-time for chloroplasts was 32 min which emphasizes the remarkable stability of the digitonin/Triton O_2-evolving PS II. O_2-evolving PS II preparations aged in the dark at 23°C but not in contact with the rate electrode gave much longer inactivation half-times, of the order of 14 h (data not shown). Similar stability results (off the rate electrode) were observed by Kuwabara and Murata [22] with a different type of O_2-evolving PS II preparation. The enhanced stability may well be due to elimination of proteolytic enzymes present in the chloroplast preparation. In contrast, the decay half-time (on the rate electrode) of the Triton preparation was about 25 min [10]. Recent results suggest an even shorter half-time, perhaps due to damage caused by long exposure to Triton.

The curious observation that Y_{19} increases with time before decreasing (Fig. 3) can be explained by the fact that the number of active O_2-evolving centers decreases with time (as seen above) while the acceptor plastoquinone pool on the reducing side of PS II remains constant (it is much more stable). If, in the O_2-evolving PS II preparation, a plastoquinone acceptor pool is common to several PS II reaction center/O_2-enzyme systems, it will take more flashes to fill the pool when some of the O_2-evolving photosystem complexes are inactivated. Thus, the relative increase in Y_{19} during the course of inactivation is a consequence of an increase in the plastoquinone/O_2-evolving enzyme ratio, due to the greater fragility of the O_2-evolving enzyme. Similar results were observed with the Triton preparations but not shown in Ref. 10. This behavior is not seen with chloroplasts because of the higher plastoquinone/O_2-evolving enzyme ratio and the fact that plastoquinone is not limiting (due to PS I function).

S_3 deactivation

The results depicted in Figs. 4 and 5 with O_2-evolving PS II preparations are, in general, consistent with the S-state model of Kok et al. [9] used to explain O_2 evolution in algae and chloroplasts. However, there are several observations indicating differences in the detailed mechanism. For example, S_3 deactivation in O_2-evolving PS II appears to be first order instead of second order as usually observed in algae or chloroplasts [3,4,19], and the decay half-time is longer.

Second-order deactivation of S_3 has been explained in terms of a requirement for an electron donor R [4], H^+ [23], and D_1 to D_3 where D_2 may be the reduced form of the DCMU-binding site, and D_1 and D_3 are unknown [19]. On the other hand, 'quasi-first-order' decay of S_3 in *Chlorella* has been reported under specialized conditions where an electron donor D_4 (tentatively identified as the reduced plastoquinone pool) was present in much greater quantity than S_3 [19]. In addition, Kok et al. [9] have suggested that the forward reaction $S_2 \rightarrow S_3$ might be first order. The isolation of O_2-evolving PS II samples exposes the O_2-evolving system to a different environment than that observed with chloroplasts. The results of Fig. 2 which show a lower pH optimum for O_2-evolving PS II * (see also Refs. 8 and 10) are consistent with this idea. Changes in the conformation of surface proteins due to the O_2-evolving PS II isolation procedure could well affect the properties, and thus the deactivation kinetics, of the O_2-evolving enzyme. For example, if H^+ [23] were required for S_3 deactivation, it might well have been available to the O_2 enzyme in larger concentrations with the O_2-evolving PS II preparations than with algae or chloroplasts (thus changing a second- to a quasi-first-order reaction). Observations of deactivation at different pH values could address this possibility. Controlled changes in the effective plastoquinone pool size could also probe this hypothesis [19].

The longer deactivation time of S_3 in the O_2-evolving PS II is explainable in terms of the observation of Joliot et al. [3] that deactivation is slower in chloroplasts than algae. An O_2-evolving PS II preparation extracted from broken chloroplasts is further still from the in situ environment in terms of unknown factors controlling deactivation.

* The pH phenomenon was predicted by luminescence studies in *Chlorella* [23,24].

S_2 deactivation

Deactivation of S_2 can be observed in Fig. 4B (Y_2) without interference from S_3 (Y_1 is very small in this case) and in Fig. 4A with potential contribution from S_3. If one subtracts the slow decay component in each case, the rapid component decays with first-order kinetics, and the half-times observed are 17 and 23 s, respectively (see Fig. 5). Joliot et al. [3] reported that S_2 deactivation was close to first order in both *Chlorella* and spinach chloroplasts. In actuality, they also show biphasic decay of S_2 in chloroplasts (Fig. 4 of Ref. 3) with half-times of about 12 and 69 s. The fact that the second component of Y_2 in Fig. 4B appears stable is of some concern. One might suspect that a substantial double-hit phenomenon could lead to the presence of significant amounts of S_0 and S_1 in addition to S_2 in Y_2. This could occur due to interference generated by the tail of our flash source, but calculations of γ (probability of double hits [18], β in the terminology of Ref. 4) using chloroplasts and our flash source result in values of 0.04–0.05. This is strong evidence against a substantial, 'normal' double-hit phenomenon, but does not rule out a special double-hit effect occurring only during the first few flashes of a sequence [25] in O_2-evolving PS II preparations. The fact that we could measure a half-time of 265 s in another sample probably means that the deactivation of the second component is variable but long.

S_2 and S_3 deactivation pathways

Deactivation in chloroplasts occurs predominantly by the 'one-step' pathway $S_3 \rightarrow S_2 \rightarrow S_1$ [3,4]. However, this does not appear to be the case for O_2-evolving PS II samples. Assuming that $S_3 \rightarrow S_2$ is rate limiting in an $S_3 \rightarrow S_2 \rightarrow S_1$ pathway, one would expect S_2 (Y_2 in Fig. 4A) first to fall off rapidly then decay with kinetics similar to those of S_3 (Y_1 in Fig. 4A). Since the second condition is not met, the assumed pathway is not valid. If $S_2 \rightarrow S_1$ were rate limiting and assuming that we are dealing with irreversible deactivation reactions, then S_2 (Y_2 in Fig. 4A) should reach a transient maximum before decaying. Since this obviously is not true, S_2 deactivation appears to be indepen-

dent of S_3 deactivation in our O_2-evolving PS II preparations.

S_0 and S_1

Except for the fate of the long-lived S_2 component, the results in Fig. 4A with O_2-evolving PS II preparations are consistent with the S-state model for O_2 evolution in algae and chloroplasts outlined in the Introduction. Fig. 6 provides further evidence for the model, since the ratio of both Y_4/Y_3 and Y_8/Y_7 in O_2-evolving PS II preparations increases sharply after three preflashes. The same results have been observed with chloroplasts, and the phenomenon has been explained in more detail [4,9] taking into account the effects of misses and double hits. Needless to say, these data show that, as in chloroplasts, the dominant route for the formation of S_0 in O_2-evolving PS II is the photochemical step $S_3 \rightarrow S_0$ (through the short-lived S_4). In addition, it demonstrates the stability of the S_1/S_0 ratio (at least for 10 min) once it has been set by flash pretreatment and deactivation. Although it is possible that there is a slow $S_1 \rightarrow S_0$ deactivation, the fact that Y_3 is always the maximum flash yield (no preillumination) even after 105 min of dark adaption (Fig. 3) argues that such deactivation is minimal.

Conclusion

The S-state model developed by Kok and coworkers [9] for algae and chloroplasts is valid for describing the general features of O_2 evolution in active, O_2-evolving PS II preparations. However, there are some differences observed in the deactivation process, particularly the apparent stability of about half of S_2 and also the dark deactivation pathway of S_3. Whether these are attributable to the differences in the way the observations were made, damage to the O_2-enzyme system during PS II isolation, or to differences resulting from changes in the environment to which the enzyme is exposed, remains to be determined.

Note added in proof (Received March 28th, 1983)

Last year we reported O_2-rate-electrode sequences that show O_2 on the first flash [10]. This work was done using the preparation of Berthold et al. [8] with market spinach obtained during the winter. We have confirmed this observation with this past winter's spinach. However, preparations according to Berthold et al. made with summer spinach do not give O_2 on the first flash, and preparations according to Yamamoto et al. [12] have never given O_2 on the first flash. In addition, we have now measured the acceptor pool of the preparation of Yamamoto et al. by fluorescence induction techniques and observe a pool size of 6–7 electron equivalents (Seibert, M., Lavorel, J. and Briantais, J.-M., unpublished results). Chloroplasts give values of 10–15 electron equivalents by this technique. Finally, we have found significant P-700 contamination in the preparation of Yamamoto et al. [12]. Room-temperature EPR studies show that 30–50% of the normal amount of P-700 found in chloroplasts is still present in the PS II preparation. However, this fact does not affect the results of this paper because electron transport between the two photosystems does not occur.

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