Patterns of oxygen emission from active oxygen-evolving photosystem II particles subjected to sequences of flashes

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1. INTRODUCTION

Preparative procedures yielding active PS II, oxygen-evolving particles have been described. The availability of such material will probably contribute decisively to the unraveling of the still poorly understood mechanism of water oxidation and oxygen emission in photosynthesis. Here we describe new patterns of oxygen emission resulting from a sequence of short flashes on 2 types of OES II (oxygen-evolving system–PS II) particles. The particles were prepared either from chloroplasts according to [1] or from *Phormidium laminosum* membrane fragments according to [2,3].

2. MATERIAL AND METHODS

Chloroplasts from market spinach were prepared in high-salt buffer as in [4]. cp-OES II particles were obtained after Triton X-100 extraction as in [1], except that in most cases the chloroplasts were treated with detergent only once (25 mg Triton X-100/mg chl; Babcock, personal communication).

Phormidium laminosum (strain OH-1-p.C1 1) was grown in medium D of [5]. Membrane fragments were prepared as in [3] which included lysozyme digestion of the cell filaments and osmotic shock of the resulting sphaeroplasts. pho-OES II particles were obtained by LDAO extraction and Sepharose 6B chromatography [2]. Under our conditions, we found that the optimal LDAO/Chl ratio was 5.2–5.3 (w/w), instead of 3.5 as reported in [2].

Hill reaction activity was measured at 25°C with a Clark-type O_2 electrode (YSI Model 5331, Yellow Springs) with 2,6-dimethylbenzoquinone and potassium ferricyanide as acceptors [2]. O_2 yields from sequences of 2μs Xenon flashes utilizing a modified PRA Model 6100B (London, Ontario) flash source were detected at room temperature (~23°C) with a Joliot-type O_2 rate electrode as in [6]; no electron acceptor was provided. The amperometric signal was differentiated electronically, and the amplitude of the leading spike of the resulting signal was taken as proportional to the O_2 yield.

LDAO (30%, w/w) was a gift from the Onyx Chemical Co. (Jersey City) and Triton X-100 was purchased from the Sigma Chemical Co. (St Louis).

3. RESULTS

Fig.1 shows typical Y_n patterns (Y_n, O_2 yield at flash number n) which we consistently observed with cp-OES II at pH 6.1. The phase of the oscillations is similar to that in control spinach chloroplasts (1st maximum at Y_3, 2nd maximum at Y_8). However, the following differences are noted:

(i) Oxygen evolution is always detected at the 1st and 2nd flash and $Y_1 > Y_2$;

(ii) Strong damping develops abruptly at the second maximum (the 3rd maximum is barely visible) followed by a steady decrease in the Y_n -values.

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Abbreviations: PS II, photosystem II; cp-OES II, O_2-evolving PS II particles isolated from chloroplasts; pho-OES II, O_2-evolving PS II particles from *P. laminosum;* chl, chlorophyll; LDAO, lauryldimethylamine oxide; DCMU, 3-(3,4dichlorophenyl)-1,1-dimethylurea

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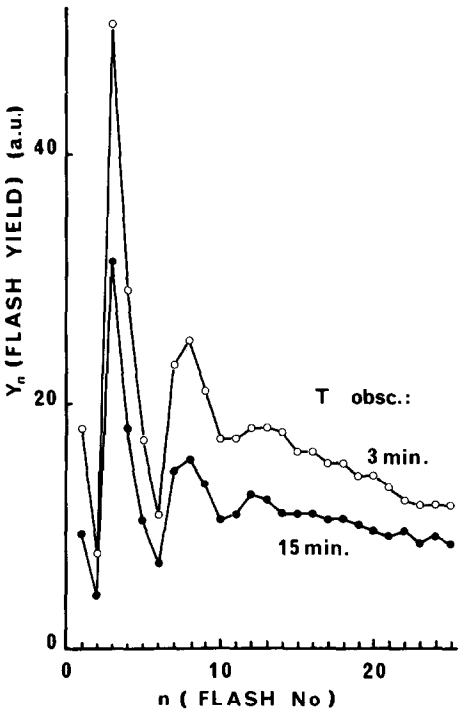

Fig.1. Effect of dark incubation on O_2 yield, Y_n (n = flash number) sequences of cp-OES II particles: flash period, 1.5 s; $T_{obsc.}$, time in darkness before the first flash sequence; (○) 3 min; (●) 15 min. Circulating medium: 15 mM $MgCl_2$, 15 mM $NaCl_2$, 20 mM Hepes (pH 6.1); sample, 3 mg chl/ml, 23°C.

A priori the first property suggests an exceedingly slow deactivation of states S_3 and S_2. The above feature was observed in spite of every precaution taken to minimize light exposure during deposition of the sample on the platinum electrode and even after an extended dark-adaptation period (fig.1; also comparison of the 2 homothetic sequences shows that the lifetime of activity in darkness at room temperature is ~25 min). S_3 deactivation was studied using the '1 2 Δt 3' classical protocol [7] and fig.2 shows that the half-time of deactivation is ~400 s, considerably longer than that in chloroplasts. Note also that the deactivation kinetics is close to a log(t) law.

That Y_1 reveals a metastable S_3 state is indicated by the fact that Y_5 is relatively higher in cp-OES II than in chloroplasts. Similarly, addition of DCMU (2×10^{-5} M) before the first flash sequence results in O_2 emission on only the first flash; Y_1 is ~60% of the control without DCMU. A problem related to the origin of Y_1 is that of the homogeneity of the preparation – the anomalous Y_1 and Y_2 might originate from a population of particles distinct from those giving the remaining part of the sequence. On the one hand, 2 observations favor heterogeneity. Preparations obtained after 2 Triton washes show a prominent Y_1, with much less O_2 emitted on subsequent flashes. When testing the same sample (1 wash) on consecutive days (kept at 0°C before testing and −80°C during overnight storage), we observed that the ratio of Y_1 to Y_3 increases during storage without appreciable loss of overall activity (e.g., $Y_1/Y_3=0.15$ immediately after preparation and 0.4 after 4 days). On the other hand, if the population is homogeneous, the fact that $Y_1 > Y_2$ is at variance with known deactivation in normal systems where the decay of S_3 is always faster than that of S_2 [7]. At any rate, it is clear that the detergent treatment has profoundly affected the S_3 deactivation mechanism and probably also the S-state distribution in the dark.

The early onset of damping during the flash sequence (fig.1) suggests that the plastoquinone pool

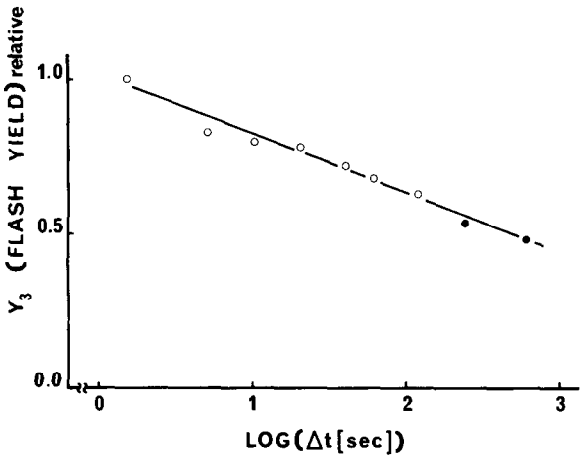

Fig.2. Kinetics of S_3 deactivation in cp-OES II particles. (Note logarithmic time scale of the abscissa.) S_3 is monitored by Y_3, the O_2 flash yield Δt s after 2 preliminary flashes spaced 1.5 s apart. Each point corresponds to a fresh sample kept in darkness on the electrode for 3 min.
Fig.3. Effect of pH on O_2 yield Y_n of cp-OES II particles: (○) pH 6.1; (●) pH 7.5; same conditions as in fig.1 but different preparation; sample, 1 mg chl/ml.

is significantly smaller than in control chloroplasts. The reoxidation of the photochemically reduced pool in the dark is quite sluggish. For example a second flash sequence following the first after 150 s darkness is similar to the first, except for a greater damping and a reduced amplitude of the Y_n-values (not shown). Comparison of the 2 sequences shows that, aside from the obvious effect of incomplete deactivation, only ~30% of the reduced pool has been reoxidized during the 150 s dark period. Deactivation of S_3 does not depend on the extent of reduction of the pool; it is not significantly faster after $n > 2$ (up to $n = 20$) flashes than after $n = 2$ flashes, as above.

We have confirmed the observation in [1] that, contrary to chloroplasts, the cp-OES II particles exhibit a higher Hill activity at acid pH (6.1) than at neutral pH (7.5), e.g., we have observed an activity of 133 μmol $O_2 \cdot h^{-1} \cdot$ mg chl^{-1} at pH 7.5 and 243 μmol $O_2 \cdot h^{-1} \cdot$ mg chl^{-1} at pH 6.1. The flash-induced O_2 emission is even more sensitive to pH (fig.3). For the same preparation, the ratio Y_3 (pH 6.1) to Y_3 (pH 7.5) is ~5; in addition, the Y_n sequence is damped to a greater extent at neutral than at acid pH. Evidently, the Hill reaction assay is mostly indicative of a limiting dark reaction (light saturation), whereas the O_2 flash yield must reflect the system II photochemical yield or the rates of the reactions on the donor side which are coupled to it. The above results are consistent the idea that the OES normally requires an acid environment for its optimal functioning (see [8,9]).

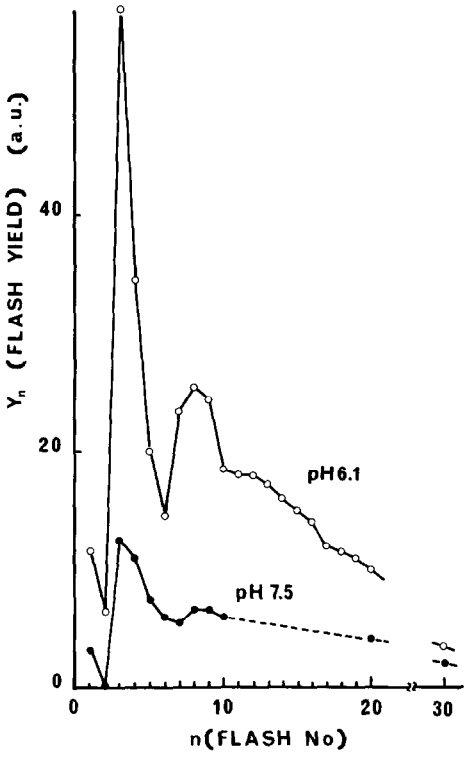

Fig.3. Effect of pH on O_2 yield Y_n of cp-OES II particles: (○) pH 6.1; (●) pH 7.5; same conditions as in fig.1 but different preparation; sample, 1 mg Chl/ml.

The Y_1, Y_2 anomaly has also been observed with the pho-OES II particles. On a chl basis, their Y_n amplitudes are smaller than that of the cp-OES II particles. Detailed examination of the amperometric signals show that this difference reflects in part a slower rate of apparent O_2 emission during the dark period following a flash; in turn, this might arise from different diffusion paths of O_2 in the 2 types of samples.

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REFERENCES

[1] Berthold, D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 134, 231–234.
[2] Stewart, A.C. and Bendall, D.S. (1979) FEBS Lett. 107, 308–312.
[3] Stewart, A.C. and Bandall, D.S. (1980) Biochem. J. 188, 351–361.
[4] Robinson, H.H. and Yocum, C.F. (1980) Biochim. Biophys. Acta 590, 97–106.
[5] Castenholz, R.W. (1970) Schweiz. Z. Hydrol. 32, 538–551.
[6] Lavorel, J. and Lemasson, C. (1976) Biochim. Biophys. Acta 430, 501–516.
[7] Forbush, B., Kok, B. and McGloin, M.P. (1971) Photochem. Photobiol. 14, 307–321.
[8] Joliot, P. and Joliot, A. (1980) Plant Physiol. 65, 691–696.
[9] Lavorel, J. (1980) Biochim. Biophys. Acta 590, 385–399.