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Reactivation of oxygen evolution of NaCl-washed Photosystem-II particles by Ca^{2+} and/or the 24 kDa protein

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We have studied the conditions required to reactivate oxygen evolution in NaCl-washed Photosystem-II particles. Restoration of oxygen evolution by Ca^{2+} revealed an heterogeneity in these Photosystem-II particles: 30% possess a low affinity site for Ca^{2+} (1–2 mM), 70% a high affinity site for Ca^{2+} (50–100 μM), even in the absence of the 24 kDa protein. The sole effect of the 24 kDa protein added back to Photosystem-II particles shortly before illumination was to stabilize oxygen evolution. Added back more than half an hour before, to Photosystem II particles at a high chlorophyll concentration, it increased oxygen evolution from approx. 40% of the control to 60–70% of the control. After reconstitution, an appreciable fraction of the low-affinity site for Ca^{2+} was still present.

Introduction

Knowledge of the mechanisms of photosynthetic oxygen evolution has considerably increased with the use of Photosystem-II enriched particles isolated from green plants. Three polypeptides of 33, 24 and 17 kDa located at the inner surface of the membrane were shown to be involved in oxygen evolution [1,2]. The 33 kDa protein maintains the four Mn atoms at their binding site [3] which may be a membranous 34 kDa protein [4,5]. Removal of the 33 kDa protein together with the 24 and 17 kDa proteins by treatments with $CaCl_2$ 1 M [6] or urea 2.6 M [3], requires 200 mM Cl^- in the medium to keep the Mn at its active site [7]. NaCl-washing removes only the 24 and 17 kDa proteins [8]. These treatments produce partially or completely inactivated PS II particles that can be more or less reactivated by Ca^{2+} [6,7,9]. A possible role for the 17 kDa protein is to concentrate Cl^- around the oxygen-evolving complex [10], a similar role for the 24 kDa protein has also been suggested [11].

Oxygen evolution of NaCl-washed PS II particles was not totally inhibited. Larsson et al. [12] suggested that it was due only to the partial removal of the 24 kDa protein rather than to a partial inhibition of all the PS-II centers. Thus the effect of NaCl washing on the functioning of the donor side of PS II has been reinvestigated in this work by studying the oxygen evolved during a train of flashes.

The 24 kDa protein also creates a high-affinity site for Ca^{2+} [13]. In its absence the Ca^{2+} concentration required to restore oxygen evolution is higher than in its presence. Others [14–16] have shown that with the readdition of the 24 kDa protein alone, reactivation of oxygen evolution occurs in NaCl-washed PS-II particles. However, discrepancies between authors still exist about the usefulness of having either the 24 kDa protein, or Ca^{2+} or both present for full reactivation of oxygen evolution. In the present report we carefully rein-

Abbreviations: PS, Photosystem; Mes, 4-morpholineethanesulphonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid.

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vestigated the conditions under which reactivation can be obtained by adding Ca^{2+} and/or the 24 kDa protein to NaCl-washed PS-II particles.

Materials and Methods

Berthold, Babcock and Yocum -type [17] Photosystem-II particles from pea chloroplasts were prepared as in Ref. 18. The average activity was 250–300 μmol O_2/mg total chlorophyll per h. NaCl washing was performed in room light as in Ref. 14. The PS-II particles were suspended in 0.3 M sucrose/10 mM NaCl/25 mM Mes (pH 6.5) at a concentration of 2–4 mg of chlorophyll/ml after their preparation. The residual activity of NaCl-treated PS-II particles varied from 10% to 40% of the total restorable activity, which corresponded to 70–80% of the activity of the intact PS-II particles. The variations in residual activity were independent of the NaCl concentration (1 or 2 M) used for the washing.

The initial rate of oxygen evolution under continuous illumination was measured with a Clark-type electrode with a response time of 1–2 s, at 20°C. The chlorophyll concentration for the experiments was 20 μg/ml. The resuspension medium in the electrode chamber was 10 mM NaCl/10 μM EGTA/25 mM Mes (pH 6.5). Phenyl-*p*-benzoquinone at 0.45 mM was used as an electron acceptor.

The 24 kDa protein was purified as in Refs. 14 and 18. It was added back to the particles at a ratio of 0.2 mg protein/mg chlorophyll. For the short incubation time, the chlorophyll concentration was 20 μg/ml; for the long incubation time, the chlorophyll concentration was 1 mg/ml.

Measurements of the oxygen evolved per flash were performed with a set-up described in Ref. 19. The flow medium was 0.3 M sorbitol/25 mM Mes 100 mM KCl (pH 6.5). For this latter experiment particles prepared according to Kuwabara and Murata [20] were used.

Results

The oxygen evolved per flash during a train of flashes (oxygen sequence) has a characteristic period 4 oscillation. Mathematical analysis of the sequences [21] allows us to determine the miss parameter $\Sigma_{i=0}^{3} \alpha$ and the dark distribution of the S_0 and S_1 states (see Ref. 22 for a review).

Fig. 1 shows a typical trace obtained with NaCl-washed PS-II particles for the residual oxygen evolution. The values of the miss parameter and the S_0, S_1 dark distribution are shown in the inset, and are very similar to those obtained with intact PS-II particles [23]. This indicates that the donor side of NaCl-washed particles which were still functional was not affected by the treatment. Nevertheless, in the absence of Ca^{2+} in the flow medium, the stability of oxygen evolution of such particles on a bare platinum electrode was dependent on illumination. In the dark the particles were very stable on the electrode, yet illumination by either a sequence of flashes or even a single flash produced an irreversible inhibition of the subsequent sequence (measured 10 min later) (not shown).

The activity of NaCl-treated PS-II particles can be restored by addition of Ca^{2+} [9] and/or the 24 kDa protein [14]. As we pointed out in the introduction, data about this subject are somewhat contradictory. Sucrose, which was present in most experiments, is contaminated by Ca^{2+} (25 μM Ca^{2+} for 0.3 M sucrose purchased from Merck). We therefore repeated the study of the reactivation of oxygen evolution for the NaCl-washed PS-II particles in a sucrose free medium. Moreover to complex traces of Ca^{2+} 10 μM EGTA was added.

Curve □ in Fig. 2 shows the restoration of

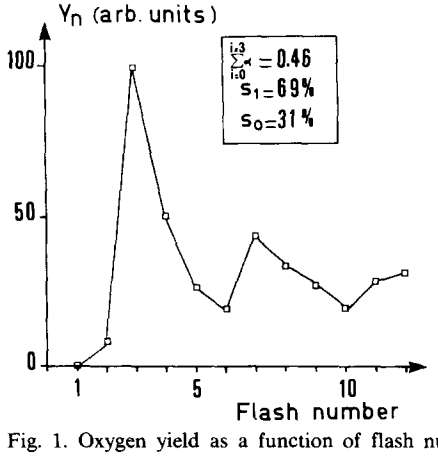

Fig. 1. Oxygen yield as a function of flash number (Y_n) for NaCl-washed PS-II particles. Flash spacing was 0.5 s. Dark adaptation 10 min on the electrode before the measurement. Chlorophyll total was 1 mg/ml.

oxygen evolution under continuous illumination by Ca^{2+} ($CaCl_2$) in NaCl-treated PS-II particles. It was possible to distinguish two ranges of Ca^{2+} concentration necessary for restoration of activity in particles which have a residual activity of 10%. The first range (50–100 μM Ca^{2+}) concerns 70% of the total restorable activity, the second (1–2 mM of Ca^{2+}), 30%. The same curve was obtained with 30 mM Cl^- present in the buffer (not shown). Thus inhibition of oxygen evolution was not due to a too low Cl^- concentration in the absence of the 24 kDa protein as in Refs. 11 and 24.

Fig. 3 shows that the half-saturation with respect to light intensity was identical for 0.4 mM Ca^{2+} or 10 mM Ca^{2+} added. The effect of the 24 kDa protein upon Ca^{2+} reactivation depends on the conditions of incubation with the NaCl-washed particles. If the 24 kDa protein is added back 1 min before the measurement with a chlorophyll concentration of 20 μg/ml, no stimulation was observed (curve ■ in Fig. 2). Under these conditions the sole effect of this protein is to stabilize

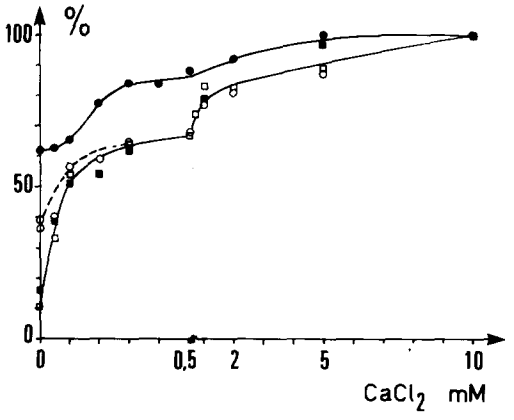

Fig. 2. Plot of the oxygen-evolution activity (in percent of the total restorable activity with 10 mM $CaCl_2$) versus $CaCl_2$ added for two samples. (a) First sample: NaCl-washed PS-II particles with (■) or without (□) the 24 kDa protein added in the medium 1 min before illumination at a protein-to-chlorophyll ratio of 1:5 (w/w) and a chlorophyll concentration of 20 μg/ml. (b) Second sample: ○, NaCl-washed PS-II particles without the 24 kDa protein; ●, the 24 kDa protein was incubated with the particles (1 mg/ml of chlorophyll) in the dark, on ice, for half an hour. The protein to chlorophyll ratio was 1:5 (w/w). The difference between the first and second sample was the residual oxygen evolution activity after NaCl washing. First sample, 10%; second sample, 35%.

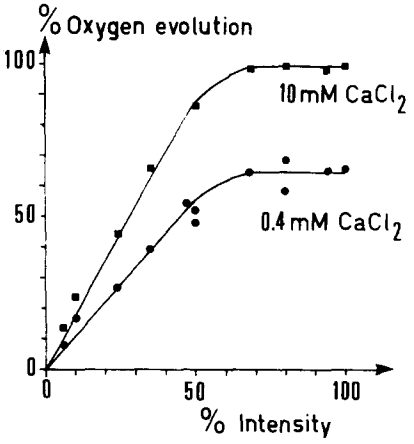

Fig. 3. Plot of the oxygen-evolution activity vs. light intensity in the NaCl-washed PS-II particles reactivated with 0.4 mM (●) or 10 mM (■) $CaCl_2$.

oxygen evolution (not shown) as observed in Ref. 24. Dekker et al. [25] suggest that this is because the protein prevents Ca^{2+} release from its site during illumination. Indeed, it seems that a single flash is sufficient to inhibit oxygen evolution in the absence of the 24 kDa protein. Therefore, when Photosystem-II centers are in the S_2 state, Ca^{2+} can be released from its binding site.

With treated PS-II particles having 40% of residual activity the curve (○) in Fig. 2 also showed two ranges of reactivation with the same two Ca^{2+} concentrations as above. The increase in residual activity from 10% to 40% seems predominantly associated with the suppression of a portion of the curve corresponding to the high affinity site for Ca^{2+}. The percent of activity restorable by 1–2 mM of Ca^{2+} was not modified. The high affinity site for Ca^{2+} appears as the least sensitive to NaCl washing.

If the 24 kDa protein was incubated for more than half an hour with the NaCl-washed particles (with 40% residual activity in this given experiment) in the dark, at a chlorophyll concentration of 1 mg/ml, on ice as in Ref. 14, in a sucrose-free medium and with 10 μM EGTA present, we then observed (curve ● in Fig. 2) a stimulation of oxygen evolution, without Ca^{2+} from 40% to 60% of the total restorable activity. It was weaker than that observed in Ref. 14. The reactivation of the remaining fraction of PS-II centers with Ca^{2+} showed that an appreciable fraction of the low-af-

finity site was still present after reactivation.

If 20 μM Ca^{2+} was present during the long dark incubation, the same curve as curve ● in Fig. 2 was obtained (not shown).

Discussion

Our results show that after NaCl washing, PS-II particles possess a residual oxygen evolution with characteristics identical to the oxygen evolution of intact PS-II particles for centers which can still function.

With NaCl-treated PS-II particles which possessed only 10% of residual activity, two ranges of reactivation by Ca^{2+} occur. For $Ca^{2+} = 0.4$ mM or $Ca^{2+} = 10$ mM we obtained the same half-activity with respect to the light intensity (Fig. 3). This result suggests that 70% of the PS-II centers have a high-affinity site for Ca^{2+} and 30% a low-affinity site for Ca^{2+}. Contrary to Refs. 13 and 18 the high affinity site exists even in the absence of the 24 kDa protein . The 24 kDa protein affected the reactivation by Ca^{2+} in two ways depending on the conditions of incubation. Shortly after its addition to NaCl-washed PS-II particles, it only stabilized oxygen evolution.

When the 24 kDa protein is present a long time before illumination, the centers that possess a high affinity site for Ca^{2+} even without this protein apparently do not need Ca^{2+} for oxygen evolution, but we cannot exclude a slow equilibrium between traces of Ca^{2+} in the medium and its site on the membrane surface.

After washing of the particles with either 1 or 2 M NaCl the residual activity varied from 10 to 40%. The reactivation of the inhibited PS-II centers by Ca^{2+} allowed us to conclude that those centers having a high affinity site for Ca^{2+}, even in the absence of the 24 kDa protein, are more resistant to the salt-treatment than those having a low-affinity site. The reasons for this remain to be elucidated.

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