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INHIBITION OF PHOTOSYNTHETIC OXYGEN EVOLUTION IN NON-VESICULAR PREPARATIONS RELEASES Mn^{2+} INTO A RESTRICTED AQUEOUS COMPARTMENT

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SUMMARY: Release of Mn^{2+} following inhibition of the water-splitting enzyme by treatment with Zn^{2+} was studied in two preparations derived from chloroplasts: oxygen-evolving Photosystem II membrane fragments and Tween-20 treated thylakoid membranes. In both cases the released Mn^{2+} was retained in the membrane fraction on centrifugation in spite of the fact that the two preparations are known to be non-vesicular. This suggests that upon inhibition Mn^{2+} is released into an aqueous compartment more restricted than the thylakoid lumen. We propose that this compartment is formed from the various proteins of the Photosystem II complex.

Several recent studies of reactions connected with photosynthetic oxygen evolution have led to the proposal that under certain circumstances ions associated with water oxidation can be trapped in aqueous compartments more restricted than the thylakoid lumen. From studies involving labelling with acetic anhydride, it was suggested that the oxygen-evolving enzyme system is situated in a special intramembranous domain, which is separated from the lumen space by a barrier which is impermeable to H^+ (1). This compartmentation of H^+ was later supported by measurements of the effect of uncouplers on flash induced pH changes (2,3).

Other workers (4) postulated that Cl^- involved in Photosystem II reactions resides in a special membrane domain. It was later concluded that the water-oxidizing enzyme is buried in depressions in the inner surface of the thylakoid membranes and exchange of H^+ and Cl^- between this compartment and the thylakoid lumen was easier in osmotically swollen chloroplasts (5). In thylakoid preparations inhibited by treatment with Zn^{2+} we found that Mn^{2+} released from the water-splitting enzyme equilibrated only slowly with the surrounding medium (6) in spite of the high permeability of the thylakoid membrane to added $Mn2^+$ (7).

As a further test of the possible compartmentation of chloroplast Mn^{2+} associated with oxygen evolution, we have investigated the release of Mn^{2+}

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by Zn^{2+} in two preparations known to consist of non-vesicular membrane fragments.

METHODS

Thylakoids from lettuce chloroplasts were prepared by the method of Nakatani and Barber (7) except that 0.33 M sorbitol, 20 mM Hepes-NaOH (pH 7.0) was used as the suspension and reaction medium.

Oxygen evolving Photosystem II membrane fragments were prepared from lettuce thylakoids by treatment with Triton X-100 in the presence of $MgCl_2$ using the method of Berthold et al. (8) as modified by Ford and Evans (9). Tween-20 treatment was done by incubating thylakoids (0.1 mg chl/ml) in the reaction medium containing 10 mM $MgCl_2$ and 1% Tween-20 for 40 min in the dark at 4°C. After centrifugation at 20 000 xg for 4 min, the pellet was resuspended in reaction medium. Zn^{2+}-treatment was done as described previously (6) except that 0.3 mg chl/ml was used.

EPR measurements were carried out as described previously (7). Quantitative values for the concentration of Mn were always determined using standards in identical media. Total Mn concentration was measured following treatment with HCl (final concentration 0.2 M).

RESULTS

We reported previously that treatment of chloroplasts with 5 mM $ZnSO_4$ causes an inhibition of the photosynthetic oxygen evolution and the conversion of manganese to $Mn(H_2O)_6^{2+}$ detectable by EPR (6). Similar results were observed with oxygen evolving Photosystem II membrane fragments prepared by Triton treatment in the presence of $MgCl_2$. The released Mn^{2+} remained associated with the chloroplast pellet on centrifugation (Table 1), and was thus not in equilibrium with the surrounding medium. In the experiment shown in Table 1 where incubation with Zn^{2+} was for 10 min, oxygen evolution was only inhibited to 44% of the control, with a correspondingly small release of Mn^{2+}.

Table 1
Distribution of EPR detectable Mn^{2+} between resuspended pellet and supernatant in Zn^{2+}-treated Photosystem II membrane fragments

	EPR-detectable Mn^{2+}			
	supernatant	pellet		acid-treated pellet
	μM	μM	nmol/mg chl	nmol/mg chl
Control	3	5	1.5	28.4
Zn^{2+}-treated	3	23	6.2	22.4

Photosystem II membrane fragments were treated with 5 mM $ZnSO_4$ or 5 mM $MgCl_2$ (control) as described in the Methods section. Immediately prior to centrifugation (18 000 xg, 4 min) the polycation polybrene was added (final concentration 1 mg/ml) to allow rapid and complete precipitation of the membrane fragments.

Incubation for 25 min under the same conditions caused total inhibition of oxygen evolution by an appreciable equilibration of the released Mn^{2+} between pellet and supernatant occurred over this longer time (results not shown). Fig. 1 shows the efflux of Mn^{2+} from the Zn^{2+}-treated pellet. The half-time for the Mn^{2+} efflux is 0.5 h, somewhat less than the value found for intact thylakoids (6). The total manganese content of the pellet declined to a constant value with a half-time of 0.5 h for the decrease (results not shown).

There seems to be a general agreement that the Photosystem II preparation obtained by Triton treatment in the presence of Mg^{2+} (8) consists of appressed sheets which are not sealed to form closed vesicles (10,11). This preparation does not contain significant amounts of the cytochrome b-f complex (8,9) so it is not possible to demonstrate access of reagents to the inner surface of the thylakoid membrane using activity measurements. To investigate the location of the released Mn^{2+} in a preparation in which we could show accessibility to both sides of the membrane, we used thylakoids incubated with the detergent Tween-20. This treatment has been shown in electron microscopical studies to produce unsealed membrane sheets, both sides of which can be labelled by cationic ferredoxin (12), and Tween-treatment is known to cause the release of plastocyanin (13). Fig. 2 shows that addition of plastocyanin rapidly restores light-induced electron transport from H_2O to methyl viologen in this preparation. Since both reduction and oxidation of plastocyanin

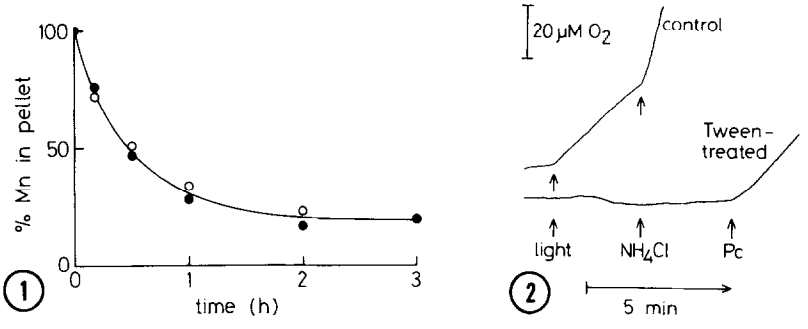

Fig. 1: Efflux of Mn^{2+} from Zn^{2+}-treated Photosystem II preparation. Oxygen-evolving Photosystem II membrane fragments were prepared and treated with 5 mM $ZnSO_4$ as described in the Methods section. Samples were taken at the times indicated and after centrifugation as described in the legend to Table 1, the pellets were resuspended and Mn^{2+} determined by EPR spectrometry. After 2 h the concentration of Mn^{2+} in the supernatant had reached the same value as that found in the resuspended pellet indicating that equilibration had taken place. The open and closed symbols represent the results of two independent experiments.

Fig. 2: Effect of plastocyanin on light-induced electron transport from H_2O to methyl viologen in Tween-treated thylakoid membranes. The membranes were suspended at 20 µg chl/ml in reaction medium containing 5 mM $MgCl_2$, 120 µM methyl viologen and 1 mM NaN_3. At the times indicated 5 mM NH_4Cl and 1.1 µM plastocyanin were added. Plastocyanin was purified from spinach as described previously (18).

normally occur at sites on the inner surface of the membrane (14), this demonstrates ready access of a large molecule to the inner surface. Zn^{2+}-treatment of the Tween preparation resulted in a 90% inhibition of the oxygen evolution and a release of Mn^{2+}. After centrifugation the amount of EPR-detectable Mn^{2+} in the pellet was 2.4 nmol/mg chl. The concentration of Mn^{2+} in the resuspended pellet was 6.5 μM while the supernatant contained only 1.1 μM Mn^{2+}, a sixfold enrichment in the membrane-containing fraction.

DISCUSSION

These results provide strong evidence for the idea that inhibition of the water-splitting enzyme with Zn^{2+} releases Mn^{2+} into an aqueous domain more restricted than the thylakoid lumen. This compartment provides a significant permeability barrier to Mn^{2+}, equilibration taking hours. It is sufficiently large to accomodate $Mn(H_2O)_6^{2+}$ in a form giving an EPR signal indistinguishable from that in aqueous solution, as first shown by Blankenship and Sauer (15) for the Mn^{2+} released following treatment with alkaline Tris buffer.

Where is this compartment located? It seems highly likely that an aqueous compartment would be associated with protein rather than being located entirely in the lipid phase of the membrane. The only major multiprotein components of the oxygen-evolving Photosystem II preparation obtained with Triton treatment are the Photosystem II reaction centre complex and the light-harvesting chlorophyll a/b protein (10). Inhibition by treatment with Zn^{2+} releases Mn^{2+} from a thylakoid membrane preparation from the cyanobacterium Anabaena variabilis and this Mn^{2+} also remains associated with the pellet after centrifugation (results not shown). This argues against involvement of the light harvesting chlorophyll a/b protein complex in the formation of the aqueous compartment. We are then left with the possibility that the various proteins of the Photosystem II complex do not form a compact unit but are arranged around a central aqueous domain. As suggested in (16) the 34 kDa protein component of the water-splitting enzyme may be involved in the formation of such a compartment. On inhibition Mn^{2+} is first released into this compartment. Following exposure to reagents such as Tris or Zn^{2+}, the Mn^{2+} remains trapped in the membrane. However other inhibitory treatments such as heating at 45°C for 10 min also cause the compartment to open with the immediate release of Mn^{2+} into the surrounding medium (17).

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