Effect of Zn^{2+} on photosynthetic oxygen evolution and chloroplast manganese

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Treatment of thylakoid suspensions with Zn^{2+} causes the appearance of an EPR signal due to Mn^{2+}. The size of the signal was linearly correlated with the inhibition of oxygen evolution. Full inhibition appeared to correspond to the release of 2 Mn atoms/reaction centre of photosystem II. The released Mn^{2+} remained associated with the chloroplast pellet on centrifugation and took several hours to equilibrate with the surrounding medium. The sequestered Mn^{2+} does not appear to be in the thylakoid interior but in a more restricted hydrophilic compartment.

Chloroplast *Thylakoid* *Manganese* *EPR* *Oxygen evolution* *Zinc treatment*

1. INTRODUCTION

A wide variety of treatments are known to inhibit chloroplast electron transport on the donor side of photosystem II. In parallel with the inhibition of oxygen evolution, manganese bound to the thylakoids is released as aqueous Mn^{2+}, giving support to the idea that manganese is involved in the oxygen evolution process.

Inhibition results both from relatively unspecific treatments which can be expected to perturb the membrane structure (such as heat, extremes of pH and chaotropic agents) and also from more specific treatments such as the addition of hydroxylamine and washing with alkaline Tris buffer [1,2] or high concentrations of divalent cations, such as Mg^{2+} [3] or Ca^{2+} [4].

In [5], treatment with Zn^{2+} over 1–5 mM inhibited oxygen evolution by isolated chloroplasts. Here, we compare the effect of Zn^{2+} on oxygen evolution and the state of chloroplast manganese as monitored by EPR spectroscopy.

2. EXPERIMENTAL

Thylakoids from lettuce chloroplasts were prepared as in [6] except that 0.33 M sorbitol, 20 mM Hepes–NaOH (pH 7.0) was used as suspension and reaction medium.

Zn^{2+} treatment was performed by incubating thylakoids (0.4 mg chl/ml in reaction medium) for 10 min in the dark at 0°C with the indicated [$ZnSO_4$]. After centrifugation for 4 min at 18000 × *g* the pellet was resuspended in a volume of the supernatant corresponding to 10% of the original. EPR measurements were done as in [6]. Quantitative values for [Mn] were always determined using standards in identical media.

The treated chloroplasts were diluted to 50 μg chl/ml in reaction medium before determining the degree of inhibition of oxygen evolution. The number of active photosystem II units was determined by measuring the extent of P700 reduction following a flash using a single beam spectrophotometer as in [7]. Light-induced oxygen evolution was measured with a Hansatech oxygen electrode in the presence of 0.5 mM phenyl-*p*-benzoquinone and 5 mM NH_4Cl.

3. RESULTS AND DISCUSSION

Fig.1 shows that treatment of a chloroplast suspension with 5 mM $ZnSO_4$ resulted in the release of Mn^{2+} in a form detectable by EPR and

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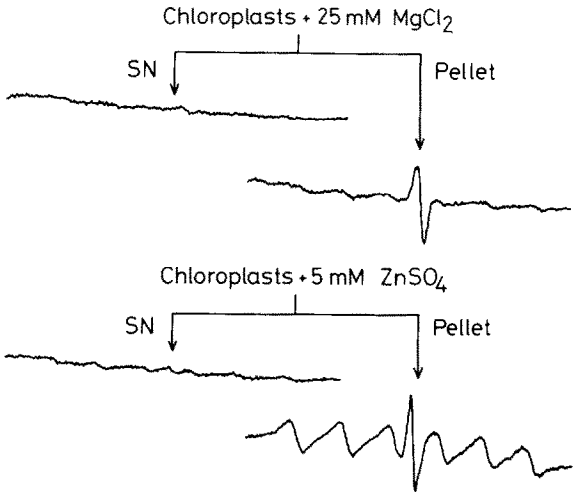

Fig.1. Effect of $ZnSO_4$ on chloroplast manganese monitored by EPR spectrometry. Thylakoids were treated with 5 mM $ZnSO_4$ as in section 2. In the control experiments, 25 mM $MgCl_2$ was added in place of the $ZnSO_4$.

spectrally identical to $Mn(H_2O)_6^{2+}$. This manganese remained associated with the chloroplast pellet on centrifugation, and was thus not in equilibrium with the external aqueous medium. Efflux of Mn^{2+} from the Zn^{2+}-treated pellet showed more complex kinetics than a simple exponential decay and was not complete even after several hours (fig.2). These results resemble those from Tris-washed chloroplasts in [8]. Efflux was not affected by the presence of the divalent cation ionophore A23187, which has in general a high affinity for Mn^{2+} [9].

The number of active oxygen-evolving centres in a partially inhibited chloroplast preparation can be estimated by measuring P700 reduction following its photooxidation by a flash. The amount of P700 reduced with kinetics corresponding to electron transfer between the photosystems is a measure of the number of electrons generated by photosystem II. Fig.3 shows that the size of the P700 signal after treatment with various [$ZnSO_4$] is inversely correlated with the amount of Mn^{2+} released in an EPR-detectable form. A similar linear relationship was found when oxygen evolution activity was measured in continuous light with phenyl-*p*-benzoquinone as acceptor (not shown). This correlation supports the idea that the manganese released by Zn-treatment is directly involved in the oxygen-evolution process.

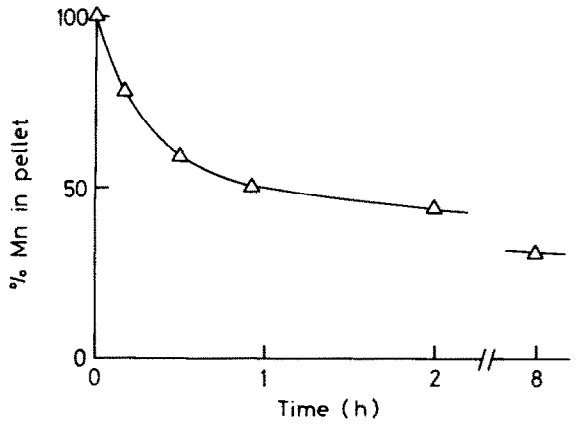

Fig.2. Efflux of Mn^{2+} from Zn^{2+}-treated thylakoids. Chloroplasts were treated with 5 mM $ZnSO_4$ as in section 2. Samples were taken at the times indicated, and after centrifugation the pellets were resuspended and Mn^{2+} determined by EPR spectrometry. The values were corrected for the amount of Mn^{2+} found in the supernatant at the time of withdrawal.

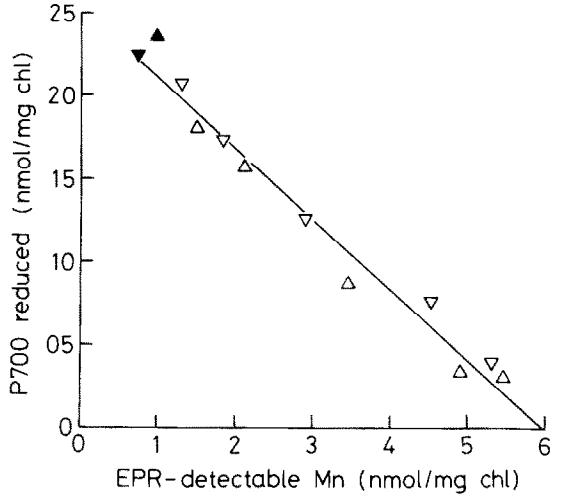

Fig.3. Relationship between EPR-detectable Mn^{2+} and amount of P700 reduced by photosystem II following treatment of thylakoids with various [$ZnSO_4$]. The P700 signal was measured at 703 nm using repetitive flash spectrophotometry and averaging the response to 20 flashes. The thylakoids were suspended in reaction medium containing 40 μM methyl viologen. [P700] was calculated from the extent of the rapid absorbance change ($t \sim 0.5$ s) using an extinction coefficient of 64 $mM^{-1}.cm^{-1}$ [14]. The different symbols are results from two experiments with different preparations of chloroplasts: (▲,▼) control with 5 mM $MgSO_4$; (△,▽) [$ZnSO_4$] from 0.5–5 mM.

Full inhibition in the experiments of fig.3 corresponded to the release of 5.0 nmol Mn^{2+}/mg chl in excess of that detectable in the control chloroplasts in the presence of 5 mM $MgSO_4$. The slope of the curve corresponds to the release of 2.4 Mn atoms for each molecule of P700 reduced by photosystem II. Since electrons are likely to be distributed between P700 and other high potential components such as plastocyanin and cytochrome *f*, this value probably overestimates the number of Mn atoms released per photosystem II reaction centre. Thus, 2 Mn released/oxygen-evolving centre is plausible. This is significantly less than ~4 Mn released/photosystem II centre reported following inhibition by Tris buffer or hydroxylamine [2].

Since the manganese liberated by Zn^{2+} is present as aqueous Mn^{2+}, it must be released into a domain which has a limited capacity for cation binding and which is surrounded by a diffusion barrier to Mn^{2+}. This compartment is not likely to be the intrathylakoid space, since the thylakoid membrane is highly permeable to added Mn^{2+} [6]. Therefore, the manganese associated with the water-splitting enzyme may not be adjacent to either the outside or the inside space of the thylakoid, but face a hydrophilic compartment into which Mn^{2+} is liberated following inhibitory treatments. This is consistent with suggestions that both H^+ [10–12] and Cl^- [13] associated with the oxygen evolution process can occur in sequestered domains where the ions are not in rapid equilibrium with the thylakoid interior.

Our results confirm the inhibitory effect of Zn^{2+} on photosystem II reported in [5] and localise the site of action to one associated with the liberation of bound manganese involved in oxygen evolution. Since treatment with 5 mM $ZnSO_4$ as described here is simple and reproducible it may find application as an alternative to the classical experimental procedures for inhibiting the water-splitting enzyme.

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