Calcium ions can be substituted for the 24-kDa polypeptide in photosynthetic oxygen evolution

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Photosystem II particles were prepared from spinach chloroplasts with Triton X-100, and treated with 1.0 M NaCl to remove polypeptides of 24 kDa and 18 kDa and to reduce the photosynthetic oxygen-evolution activity by about half. Oxygen-evolution activity was restored almost to the original level with 10 mM Ca^{2+}, in a similar manner to the rebinding of 24-kDa polypeptide. Other cations such as magnesium, sodium and manganese ions could not restore any oxygen-evolution activity. These observations, together with a kinetic analysis, suggest that Ca^{2+} can be substituted for the 24-kDa polypeptide in photosynthetic oxygen evolution in Photosystem II particles.

Ca^{2+} *24-kDa polypeptide* *Oxygen evolution (Spinach chloroplast)* *Photosystem II* *Photosynthesis*

1. INTRODUCTION

Recent investigations [1–11] have suggested that 3 membrane-bound polypeptides of 33, 24 and 18 kDa are involved in the photosynthetic oxygen-evolution system of PS II membrane preparations [1–10] and cholate-treated thylakoid membranes [11]. Treatment of PS II preparations with concentrated NaCl specifically released the 24-kDa and 18-kDa polypeptides and partially inactivated oxygen evolution [2,3,9,10]. Both polypeptides can rebind to the membranes to their original levels [3]. The rebinding of 24-kDa polypeptide reactivates oxygen evolution, whereas that of 18-kDa polypeptide has no effect on oxygen-evolution activity [3,9,10].

There are contradicting views on the function of the 24-kDa polypeptide. We have suggested that this polypeptide is a regulatory factor in oxygen evolution, since oxygen can be evolved in PS II particles completely depleted of it [2,3]. On the other hand, authors in [10] have claimed that it is essential for oxygen evolution. In this study, we investigated the effect of various salts on oxygen evolution of PS II particles and found that Ca^{2+} could be substituted for the 24-kDa polypeptide. We suggest that the 24-kDa polypeptide plays a regulatory role in oxygen evolution.

Abbreviations: Chl, chlorophyll; Mes, 2-(*N*-morpholino)ethanesulphonic acid; PS II, Photosystem II

2. MATERIALS AND METHODS

PS II particles were prepared from spinach chloroplasts with Triton X-100 as in [1] and stored in liquid nitrogen [3]. The PS II particles were treated with 1.0 M NaCl at pH 6.5 as in [3] to remove all 24-kDa and 18-kDa polypeptides. They were washed with and resuspended in 300 mM sucrose, 10 mM NaCl and 25 mM Mes–NaOH (pH 6.5) (medium A). The particles treated with medium A instead of 1.0 M NaCl are designated as the untreated PS II particles. All the above procedures were performed at 0–4°C.

The NaCl-treated and untreated particles were incubated at 25°C for 2 min in medium A containing a designated concentration of salt, and then their oxygen-evolution activity was measured at

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25°C with a Clark-type oxygen electrode in the presence of 0.3 mM phenyl-*p*-benzoquinone and 0.05% bovine serum albumin [1]. Chlorophyll concentration was determined as in [3].

3. RESULTS AND DISCUSSION

Treatment of the PS II particles with 1.0 M NaCl reduced oxygen-evolution activity by about half as in [3]. The oxygen-evolution activity of NaCl-treated particles was restored to about 90% of the original level with 10 mM $CaCl_2$ (fig.1). The activity of the untreated particles was almost unaffected by this salt.

Table 1 shows the effect of various salts on oxygen-evolution activity of untreated and NaCl-treated particles. $CaCl_2$ and $Ca(NO_3)_2$, but neither $MgCl_2$ nor NaCl, stimulated oxygen-evolution activity of the NaCl-treated particles. This suggests that the oxygen evolution of NaCl-treated particles is stimulated specifically by Ca^{2+}. $MnCl_2$ was rather inhibitory in both untreated and NaCl-treated particles.

Fig.2A shows the effect of NaCl treatment and

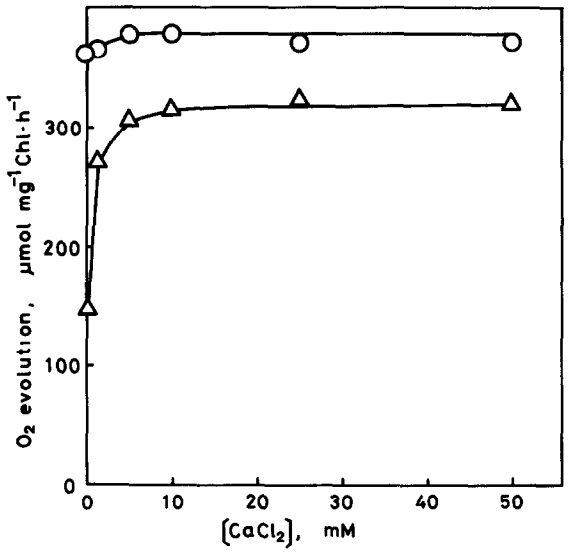

Fig.1. Effect of $CaCl_2$ on oxygen-evolution activity in untreated and NaCl-treated PS II particles. Various concentrations of $CaCl_2$ were added to the reaction mixture, and after 2-min incubation at 25°C oxygen evolution was measured at a light intensity of 660 $W \cdot m^{-2}$. (○—○) Untreated particles, (△—△) NaCl-treated particles.

Table 1

Effect of various salts on oxygen-evolution activity of untreated and NaCl-treated PS II particles

Salt added		O_2 evolution ($\mu mol \cdot mg^{-1}$ Chl $\cdot h^{-1}$)	
		Untreated	NaCl-treated
None		340	160
$CaCl_2$	(5 mM)	370	290
$Ca(NO_3)_2$	(5 mM)	350	240
$MgCl_2$	(5 mM)	330	170
$MnCl_2$	(5 mM)	260	120
NaCl	(10 mM)	330	160

Oxygen-evolution activity was measured in the presence of various salts as in fig.1

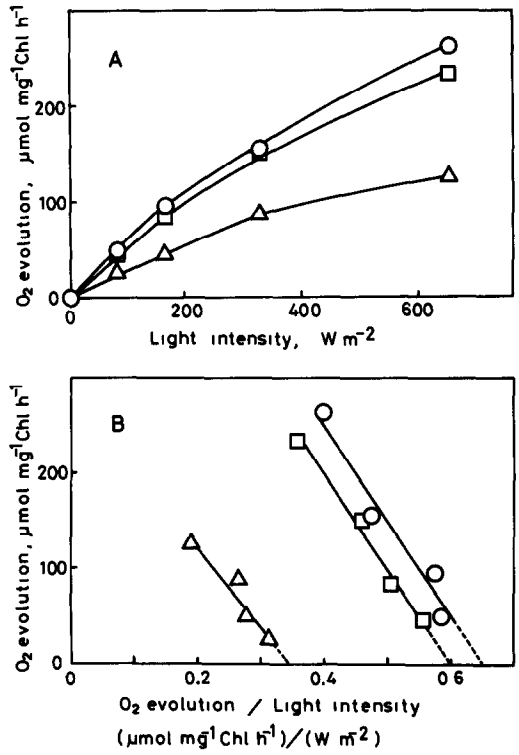

Fig.2. Effect of NaCl treatment and $CaCl_2$ upon the light-intensity dependence of oxygen-evolution activity. The light intensity was varied with neutral density filters. (A) Plot of oxygen-evolution activity *vs* light intensity. (B) Plot of oxygen-evolution activity *vs* the activity divided by light intensity. (○—○) Untreated particles in the absence of $CaCl_2$, (△—△) NaCl-treated particles in the absence of $CaCl_2$, (□—□) NaCl-treated particles in the presence of 5 mM $CaCl_2$.

Table 2

Effect of NaCl treatment and $CaCl_2$ on the kinetic parameters for oxygen evolution of PS II particles

Type of particles	Salt added	K'_m ($W \cdot m^{-2}$) ($\times 10^2$)	ϕ_{rel}	V_{max} ($\mu mol \cdot mg^{-1}$ Chl $\cdot h^{-1}$)
Untreated	None	10.1	1.00	660
NaCl-treated	None	8.2	0.54	290
NaCl-treated	5 mM $CaCl_2$	9.7	0.93	590

Values were taken from the plots in fig.2B. The relative quantum yield is normalized to the value of the untreated PS II particles in the absence of $CaCl_2$

Ca^{2+} on the light-intensity dependence of oxygen-evolution activity. The NaCl treatment decreased the activity to about half, and 5 mM $CaCl_2$ restored it at both high and low light intensities. Fig.2B shows plots of oxygen-evolution activity *vs* activity divided by light intensity. Each plot can be approximated by a straight line of similar slope to the other. If the plots can be analyzed in a way similar to enzyme kinetics, the slope, abscissa intercept and ordinate intercept of the line correspond to the apparent Michaelis constant (K'_m), relative quantum yield (ϕ_{rel}) and maximum rate (V_{max}), respectively. These parameters obtained from fig.2B are presented in table 2. K'_m was relatively constant throughout the inactivation by NaCl and restoration by Ca^{2+}. Both ϕ_{rel} and V_{max} were reduced to about half by NaCl and almost restored by Ca^{2+}.

The restoration of oxygen-evolution activity by Ca^{2+} is very similar to that by the rebinding of the 24-kDa polypeptide, which also restores the quantum yield and maximum rate of oxygen evolution of the NaCl-treated particles almost to the original level of the untreated PS II particles. These findings suggest that Ca^{2+} can be substituted for the 24-kDa polypeptide in the oxygen-evolution system. A possible interpretation for this phenomenon is that both the 24-kDa polypeptide and Ca^{2+} interact with the oxygen-evolution system in similar ways in optimizing its configuration for oxygen evolution. We conclude that the 24-kDa polypeptide is a regulatory rather than essential factor of the oxygen-evolution system.

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