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The reactivation properties for different anions in chloride-depleted photosystem II membranes

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Introduction

In photosystem II, chloride is needed for optimum oxygen evolution. We have previously presented the one-site, two-state model [Lindberg, K. and Andréasson L. E.] for the binding of anions to chloride-depleted PS II membranes, in which switching occurs from an open state with loose binding to a closed, tight-binding state. This model is also valid when glycerol is used as a cryoprotectant with dissociation constants for chloride of 0.8 mM and 13 μM for the two states, respectively.

Materials and Methods

Oxygen-evolving PS II membranes were prepared from hydroponically grown spinach as described previously [Franzén, L.-G. Hansson, Ö. and Andréasson, L.-E.] and stored in 20 mM Mes-NaOH, 15 mM NaCl, 5 mM $MgCl_2$ with 0.4 M sucrose as a cryoprotectant. Oxygen evolution rates, extrapolated to saturating light intensities, were 700-900 μmol O_2/(mg chlorophyll·h). Cl^- -free PS II membranes were prepared by dialysis against Cl^--free Mes-NaOH buffer (Sigma Ultra). The Cl^--free Mes buffer used for washing and dialysis to prepare Cl^-depleted PS II membranes contained 1.4 M glycerol (Merck, 0.0005% Cl^-). In all buffers the residual chloride concentration, measured using the photochemical-Ag method [Lindberg, K., Vänngård, T. and Andréasson, L.-E] was below the detection limit (1 μM).

Steady-state oxygen evolution activities were measured and digitised with an oxygen electrode (Hansatech OMS1), in Mes-NaOH, pH 6.3 with appropriate salt and cryoprotectant present depending on measurement and with 0.5 mM PPBQ as an electron acceptor.

Results and discussions

The role of chloride in PS II membranes have to be considered differently depending on the protocol used for chloride removal as well as the choice of cryoprotectant. When comparing the polypeptide composition using SDS-PAGE after chloride depletion there is no difference between untreated PS II membranes and PS II membranes dialysed with glycerol as a cryoprotectant. PS II membranes, depleted of chloride using sulphate at elevated pH in sucrose, show extensive loss of extrinsic proteins and generally have a low oxygen evolution rate. However, the use of the same protocol for depletion but in glycerol will only partially remove the extrinsic 17 kDa and 23 kDa polypeptides. Upon chloride depletion of PS II membranes the rate of oxygen evolution is suppressed to different levels depending on the choice of

cryoprotectant. The rate in sucrose shows a higher degree of suppression than in glycerol. When glycerol is used as a cryoprotectant in chloride-depleted PS II membranes the demand for a reactivating anion is relatively low and all oxygen-evolving centres show an activity of 55 % of the control compared to 30% in sucrose. Also, the transfer of chloride-depleted membranes from a buffer containing glycerol to one containing sucrose will decrease the oxygen evolution rate from 55% to 30%. This shows that glycerol can compensate for some of the losses in activity caused by chloride depletion in sucrose. Although the need for chloride is less pronounced in glycerol, chloride or another anion as a co-factor is nevertheless needed for optimal oxygen evolution. To rule out the effect of the electron acceptor during steady state measurements of oxygen evolution the K_m for PPBQ was determined in glycerol with or without chloride. The values of 100 µM and 118 µM, respectively, where found, the almost equal affinity of electron acceptor suggest that the effect of chloride depletion may be localised to the donor-side. In figure 1 the water-oxidizing activities of the untreated, dialysed and reactivated PS II membranes were measured at different light intensities. The dialysis leads to a loss of activity to 55% of that of the untreated PS II membranes. The activity could be reactivated to 82% of the untreated PS II membranes by adding 10 mM of NaCl.

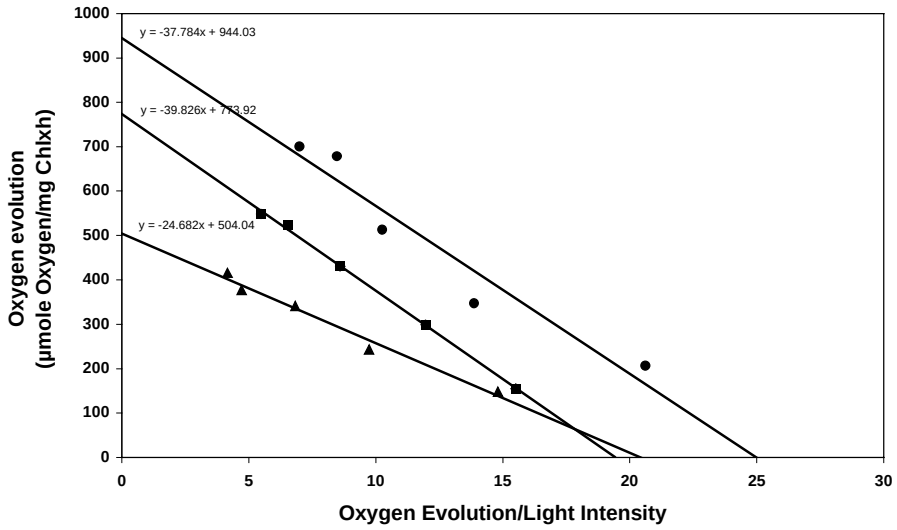

Figure 1. Oxygen evolution of PS membranes (BBY) measured by a Clark-electrode at 20° C at a chlorophyll concentration of 20 µg ml^{-1}. (●) Control BBY membranes in supplemented with 5 mM MgCl, 15mM NaCl (▲) Chloride-depleted BBY membranes in chloride free buffer. (■) Chloride-depleted BBY membranes reactivated with 10 mM NaCl. All samples where measured in 20 mM MES-NaOH buffer, pH=6.3, and 1.4 M glycerol.

The common point of interception on the abscissa axis shows that all centres are affected by the reactivation. The difference between reactivated and untreated membranes indicates that a minor fraction of the centres (about 20 %) has been irreversibly inactivated by the depletion treatment.

Chloride-depleted PS II membranes respond differently to the anions Cl^-, Br^-, NO^-_3, I^- and F^-. Chloride and bromide reactivate chloride-depleted PS II membranes in a similar way with a conversion from an open state with weak binding to a closed, tight-binding state (table 1). Iodide serves as a good substitute for chloride with comparable oxygen evolution rates but activation is only achieved in PS II

membranes incubated for a long time (2 h). If iodide is incubated for a short time, typically 10-15 s, it will act as an inhibitor. The inhibition by I^- shows a biphasic curve, which may indicate the presence of two separate binding-sites (Fig. 2).

Table *1.* All measurements was done in 20 mM MES-NaOH buffer at pH=6,3. To determine the dissociation/inhibitory-constants, the activity was measured immediately (15 s) after addition of the membranes to the anion-containing assay medium or the activity was measured in anion-free medium after incubation with anion for 2 h.

Anion	**K_d (mM)** Short incubation time	**K_d (µM), (mM)*** Long incubation time	**K_i (µM)*, (mM)** Short incubation time	**K_i (mM)** Long incubation time
NaCl	0.8	13		
NaBr	2.8	31		
NaI		13	7*, 10.5	
$NaNO_3$	0.8	2.8*		
NaF			17	14.5

Obviously the conversion from loose to a tight binding prevents I^- deactivation. It is possible that I^- initially binds in a position where is it oxidised with the resulting iodine serving as an inhibitor by iodinating protein residues. Iodination has earlier been observed in Cl^--depleted PS II [Ikeuchi, M., Koike, H. and Inoue Y.]. Prolonged incubation with I^- then, as with Cl^-, leads to a switch to a high-affinity binding where the site may be displaced from its initial position or otherwise change so that bound I^- can no longer be oxidised. One should not, however, exclude the possibility of two separate mutually exclusive binding sites for I^-.

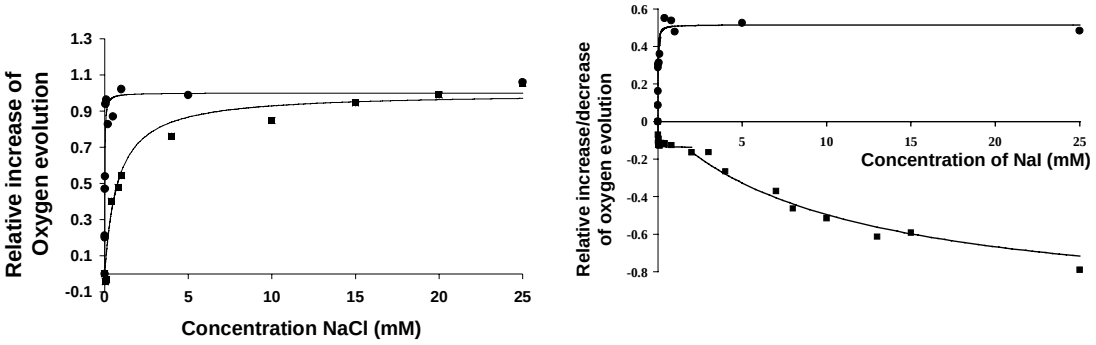

Figure 2. Anion-dependent (Cl^-, I^-) recovery of oxygen-evolution in chloride-depleted PS II membranes. PS II membranes were dialysed against anion-free buffer for 5 h in darkness. (■) The activity was measured immediately (15 s) after addition of the membranes to the ion-containing assay medium; (●) the activity was measured in a chloride-free medium after incubation with the ion for 2 h at the concentrations indicated in the figure.

NO^-_3 is a poor substitute for chloride and reactivation gives less then half the oxygen evolution rate compared to short-time Cl^- reactivation. If $NO3^-$ is incubated for long time only marginal reactivation is achieved accompanied with a weakening of binding. In similarly with I^- but in contrast to Br^- and Cl^-, NO_3^- seems to affect PS II differently depending on its mode of binding. This suggests significant alterations in the binding site occur in connection with binding-change switch.

References

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