Commentary

On the role of water-soluble polypeptides (17, 23 kDa), calcium and chloride in photosynthetic oxygen evolution

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Received 13 August 1985

Contrary to the assertion by Preston and Critchley [(1985) FEBS Lett. 184, 318–332] that there is no correlation between calcium and water-soluble 23 and 17 kDa polypeptides in promoting oxygen evolution activity, it can be shown that both calcium and the polypeptides are required for activity under physiological conditions. In the absence of the two polypeptides, non-physiological concentrations of both calcium and chloride must be present for activity; neither ion can, by itself, promote high rates of oxygen evolution.

Photosystem II *Polypeptide* *Oxygen evolution* *Calcium* *Chloride*

A substantial body of experimental evidence supports the view that the oxygen-evolving complex of photosystem (PS) II is comprised in part of manganese, chloride and calcium; these inorganic cofactors, uniquely arranged in a proteinaceous environment, catalyze the sequential 4-electron oxidation of water to molecular oxygen. It is now widely believed that 3 water-soluble polypeptides (33, 23 and 17 kDa) form part of the structure of the oxygen-evolving apparatus. The 33 kDa species is in some way associated with manganese binding to the PS II complex, although under certain conditions the polypeptide can be removed from the membrane without concurrent release of manganese [1–3]. For the 17 and 23 kDa water-soluble proteins, our observations [4] indicate that these species are required for high-affinity binding of calcium within the oxygen-evolving complex. Boussac and Etienne [5] have shown that the 23 kDa protein is in fact required for high-affinity binding of Ca^{2+}. Even though our experimental data suggest that the 17 and 23 kDa species create an environment which promotes tight binding of Ca^{2+}, there is no evidence that these polypeptides directly bind calcium. Speculating on this effect, we suggest that the 17 and 23 kDa polypeptides might affect the structure of a putative Ca^{2+} receptor protein to favor tight binding of the ion, alternatively, the 17 and 23 kDa species may form part of a structural domain which concentrates calcium. A structural role for the 17 and 23 kDa polypeptides has been further demonstrated by our own observations regarding the accessibility of the Mn complex to exogenous reductants [6], as well as by the observations of Akabori et al. [7], Andersson et al. [8] and Miyao and Murata [9], who have proposed that the 17 and/or the 23 kDa species affect the optimal concentration of Cl^- required for oxygen evolution activity.

Table 1 shows the effect of calcium and various anions on the oxygen evolution activity of a salt-washed PS II preparation which was also depleted of Cl^- by extensive dialysis against NaF (10 mM). It is clear from table 1 that calcium and chloride are both required for an active oxygen-evolving complex; although Cl^- can be effectively replaced by Br^- (or to a lesser extent by NO_3^-), the cation effect is specific for calcium. Recently, Preston and Critchley [10] reported that the Ca^{2+} requirement for oxygen evolution activity is not altered by the 17 and 23 kDa species and suggested that both control and salt-washed PS II membranes require

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00145793/85/$3.30

Table 1

Effect of calcium and anions on oxygen evolution activity of Cl^--depleted high salt treated PS II membranes

Additions	% activity
None	4
NaCl (20 mM)	26
$Ca(Ac\text{-})_2$ (10 mM)	8
$CaCl_2$ (10 mM)	80
$CaBr_2$ (10 mM)	76
$Ca(NO_3)_2$ (10 mM)	46
$MgCl_2$ (10 mM)	28

Isolation of PS II membranes, free of PS I and having high rates of oxygen evolution activity, was carried out as described in [3]. The PS II complex was depleted of the 17 and 23 kDa polypeptides by treatment with 2 M NaCl (see [4]); Cl^- was replaced by F^- by dialysis of the salt-washed PS II complex against a medium containing 0.4 M sucrose, 50 mM Mes, pH 6.0, and 10 mM NaF. The PS II complex was illuminated with continuous light in a solution containing 50 mM Mes, pH 6.0, 0.4 M sucrose, the salt indicated above, 10 μg Chl/ml and 400 μM DCBQ (control activity: 720 μmol O_2/mg Chl per h)

2 mM Ca^{2+} for optimal activity. Since we have never observed a calcium effect in intact PS II membranes, unless the system was damaged during exposure to the detergent, as evidenced by lowered rates of oxygen evolution (see also [11]), we would suggest that the 2 mM Ca^{2+} requirement observed in control preparations by the authors in [10] is due to damage of the PS II complex incurred during isolation. As shown in table 2, release of the 17 and 23 kDa polypeptides is not necessarily followed by immediate release of Ca^{2+}; calcium is easily removed from the salt-washed PS II complex either by exposure to EGTA [4] or by illumination [12]. Thus, rebinding of the 2 polypeptides (17, 23 kDa) to salt-washed membranes which have not been exposed to EGTA leads to the reactivation of the PS II complex without any requirement for addition of external Ca^{2+}. In contrast, rebinding of the 17 and 23 kDa species to salt-washed, EGTA-treated PS II membranes does not restore oxygen evolution activity unless calcium is also provided. In [4] we showed that reactivation by Ca^{2+} of the 17,23 kDa reconstituted PS II complex was a

Table 2

Inhibition and selective reconstitution of PS II membranes

Experiment		Treatment	Polypeptides bound		Activity*	
			17 kDa	23 kDa	$-CaCl_2$	$+CaCl_2$
1		None	+	+	740	750
2		2 M NaCl (1 h)	–	–	190	590
3	(i)	2 M NaCl (1 h)	+	+	580	610
	(ii)	2 1-h dialysis steps against SM + 2 M NaCl				
	(iii)	2 1-h dialysis steps against SMN				
4	(i)	2 M NaCl (1 h)	+	+	240	600
	(ii)	2 1-h dialysis steps against SM + 2 M NaCl + 2 mM EGTA				
	(iii)	1 h dialysis step against SMN + 2 mM EGTA				
	(iv)	1 h dialysis step against SMN				

* The assay medium contained 50 mM Mes, pH 6.0, 20 mM NaCl ($\pm$ $CaCl_2$) and 400 μM DCBQ. All samples were incubated for 10 min in the assay medium before illumination. SM, 0.4 M sucrose plus 50 mM Mes, pH 6.0; SMN, 0.4 M sucrose, 20 mM NaCl and 50 mM Mes, pH 6.0

relatively slow process, but once the cation was bound it was impossible to remove even by treatment with EGTA. Preston and Critchley [10] have reported that rebinding of the purified 17 and 23 kDa species to a salt-washed PS II preparation partially restored activity, and that even in the presence of the polypeptides there was a requirement for 2 mM Ca^{2+}. These results do not contradict our own observations, as the authors in [10] claimed; rather, the high salt treated PS II membranes used by Preston and Critchley were not treated with EGTA before addition of the polypeptides. Moreover, the report that even intact PS II membranes required 2 mM Ca^{2+} for full activity [10], as well as the conditions of the titration with calcium (not enough time was allowed for calcium to penetrate and reactivate the polypeptide reconstituted system) (see [4]), explains the apparent requirement for a high concentration of calcium (2 mM), even in the presence of the 17 and 23 kDa species.

The fact that the 17,23 kDa polypeptide-depleted PS II complex is very active in the presence of high, non-physiological, concentrations of Ca^{2+} and Cl^- suggests that the 17 and 23 kDa proteins are not directly involved in the catalytic mechanism of water oxidation. Under physiological conditions, however, the 17 and 23 kDa polypeptides are significant structural components of the PS II complex. It has been shown that the 17 and 23 kDa proteins create an environment which 'concentrates' Ca^{2+} and Cl^-, and in addition shields the oxygen-evolving complex from exogenous reductants [4,6–10].

ACKNOWLEDGEMENTS

Supported by grants from USDA/SEA/CRGO (G.T.B. and C.F.Y.) and The National Science Foundation (C.F.Y.)

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