Short Communication

Functional and Structural Aspects of the 10 kDa and 22 kDa Polypeptides of Photosystem II*

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Higher plant photosystem II is a supramolecular complex with both integral and peripheral protein subunits. It is located mainly in the appressed region of the thylakoid membrane, in the chloroplast. The catalytic core of photosystem II appears to be made up of at least five different integral, hydrophobic proteins. In addition, three extrinsic proteins of 33 kDa, 23 kDa and 16 kDa, located at the inner side of the thylakoid membrane, are components of the oxygen-evolving complex of photosystem II (for a review see Ref. 1).

It has previously been shown, by an immunoprecipitation study, that there exists a close structural association between the 33 kDa and 23 kDa proteins, and three polypeptides of 10 kDa, 22 kDa and 24 kDa.[2] The involvement of the 10 kDa polypeptide in photosynthetic oxygen evolution was further implicated by its release from the inner side of the thylakoid membrane by treatment with alkaline Tris,[3] a classical method for inhibiting the oxygen-evolving activity of photosystem II.

The present communication reports a mild method for the specific release of the 10 kDa and 22 kDa polypeptides from the thylakoid membrane and the effects of this on the oxygen-evolving activity. Some properties of the isolated 10 kDa and 22 kDa polypeptides are also described.

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Materials and methods

Spinach thylakoid membranes were prepared as in Ref. 4, and photosystem II membranes were prepared as in Ref. 5. The photosystem II membranes were treated with (a) 1 M NaCl, 10 mM Mes (pH 6.5) or (b) 1 M NaCl, 0.06 % Triton X-100, 10 mM Mes (pH 6.5). After treatment, the photosystem II membranes were pelleted by centrifugation at 40 000 × *g* for 30 min. Oxygen evolution was measured polarographically as in Ref. 4, and protein re-binding was performed as in Ref. 6. The 10 kDa and 22 kDa polypeptides from solubilized photosystem II membranes were purified by ion-exchange chromatography in the presence of detergent. After 30 min solubilization of photosystem II membranes (5 mg chl ml^{-1}) in 10 mM sodium phosphate (pH 6.5) containing 2 % Triton X-100 and 3 % Zwittergent TM-314, unsolubilized material was pelleted by centrifugation at 150 000 × *g* for 45 min, and the supernatant was fractionated on a CM-Sepharose® column equilibrated with 10 mM sodium phosphate (pH 6.5) and 0.05 % Triton X-100. Proteins were eluted with a NaCl gradient of 0–600 mM NaCl in the equilibration buffer. The 22 kDa polypeptide eluted at 230 mM NaCl, and the 10 kDa polypeptide at 450 mM NaCl. The 23 kDa protein was isolated as described earlier.[7]

Sodium dodecylsulfate-polyacrylamide gel electrophoresis was performed as in Ref. 8, and immunoblotting was carried out as in Ref. 9. Triton X-114/water phase-partitioning was performed as in Ref. 8.

Results and discussion

In order to facilitate functional studies of the 10 kDa and 22 kDa polypeptides a mild and specific method for their release was designed. Treatment of photosystem II membranes with NaCl-Triton was found to remove 86 % of the 10 kDa polypeptide and 70 % of the 22 kDa polypeptide, as well as the extrinsic 16 kDa and 23 kDa proteins (Table 1, Fig. 1). This treatment did not, however, release the 33 kDa protein or the manganese. NaCl alone removed only the 16 kDa and 23 kDa proteins, in accordance with earlier studies.[4] However, when the isolated 23 kDa protein was re-added, it was found that the degree of reconstitution possible with photosystem II membranes devoid of the 16 kDa and 23 kDa proteins differed profoundly from that with photosystem II membranes also lacking the 10 kDa and the 22 kDa polypeptides. Even though both preparations had lost their oxygen-evolving activity, only in the NaCl treated preparation could significant (67 %) activity be restored by re-addition of the purified 23 kDa protein (Table 1). In contrast, the activity after reconstitution with the 23 kDa protein amounted to only 13 % in the NaCl-Triton treated preparation. However, high concentrations of $CaCl_2$, which have previously been shown to compensate for the 23 kDa protein,[10] restored high activities in both the NaCl treated (73 %) and the NaCl-Triton treated (64 %) photosystem II preparation. These results indicate that the catalytic centres of photosynthetic oxygen evolution remained largely intact after these treatments, but that only in the NaCl treated preparation could oxygen evolution be restored by re-addition of the 23 kDa protein. To investigate the reason for this difference, the re-binding of the 23 kDa protein to the NaCl treated and the NaCl-Triton treated preparations was examined. In the NaCl treated preparation the 23 kDa protein re-bound to 65 % of the original level, which correlates well with the 67 % restoration of the oxygen-evolving activity. In contrast, the 23 kDa protein re-bound to only 10 % of the original level in the NaCl-Triton treated photosystem II preparation, strongly indicating that it is the inability of the 23 kDa protein to re-bind to its functional site which prevents its stimulation of the oxygen-evolving activity. From these observations it is concluded that the 10 kDa and/or the 22 kDa polypeptide are necessary for the binding of the 23 kDa protein to the thylakoid membrane. The isolated polypeptides (Fig. 1) did not contain any metals or other cofactors with absorption in the visible region of the spectrum. They also appeared to be hydrophobic, since they preferred the hydrophobic Triton X-114 phase to

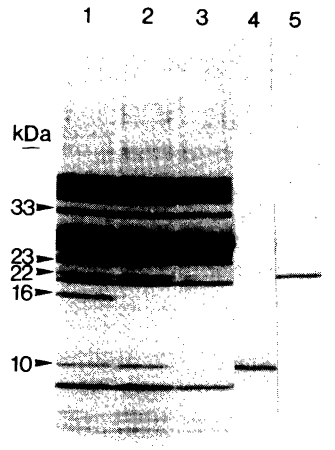

Fig. 1. Sodium dodecylsulfate-polyacrylamide gel electrophoresis of: (1) photosystem II membranes, and membranes treated with (2) NaCl or (3) NaCl-Triton. (4) Isolated 10 kDa polypeptide and (5) isolated 22 kDa polypeptide.

Table 1. Effects of treatment with 1 M NaCl or 1 M NaCl-0.06 % Triton X-100 on photosystem II membranes. Values relate to levels of the indicated proteins in untreated photosystem II membranes. 100 % oxygen evolution activity = 315 μmol O_2(mg chl)$^{-1}$ h^{-1}.

Properties	NaCl (%)	NaCl-Triton (%)
Relative amounts of proteins		
23 kDa	12	5
22 kDa	100	30
10 kDa	100	14
Rebinding of 23 kDa protein	65	10
Oxygen evolution	20	7
+ 23 kDa protein	67	13
+ 10 mM $CaCl_2$	73	64

the aqueous phase when subjected to Triton X-114/water phase-partitioning. A conclusive determination of whether these polypeptides are integral or peripheral must, however, await further analyses.

Further experimentation is also required to determine which of the 10 kDa or 22 kDa polypeptides binds to the 23 kDa protein, as well as to elucidate other functional roles of the 10 kDa and 22 kDa polypeptides.

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