Short Communication

Small Polypeptides in Oxygen-Evolving Photosystem II Core Preparations*

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Photosystem II preparations with strikingly simple polypeptide composition that are cabable of water oxidation have recently been reported.[1,2,3] These so-called photosystem II core preparations are the structurally simplest preparations so far reported which are able to perform the complete photosystem II reaction, and they contain both hydrophobic and hydrophilic polypeptide subunits. The hydrophobic constituents are the apopolypeptides of CPa_I and CPa_{II} of molecular weight 47 and 43 kDa, respectively, the 32 kDa Q_B and D2 apopolypeptides[4,5,6] and the 9 kDa apopolypeptide of the cytochrome *b*-559. In addition, a hydrophilic 33 kDa protein,[7,8,9] previously implicated in connection with the catalytic manganese, is present.

Through analysis with a sodium dodecylsulfate urea-polyacrylamide gel electrophoresis system with high resolution in the low molecular weight region, we have been able to detect, in photosystem II core preparations, several previously unreported polypeptides having molecular weights lower than the 9 kDa apopolypeptide of cytochrome *b*-559.[10] One of these is a 5 kDa hydrophilic polypeptide which has been isolated and characterized,[10] while another was found to be the newly discovered 4.3 kDa subunit of cytochrome *b*-559.[11]

Materials and methods

Spinach thylakoid membranes were prepared as previously described,[12] and photosystem II membranes were prepared according to Berthold *et al.*,[13] with modifications as in Ref. 14. Stroma lamellae vesicles were prepared as in Ref. 15. A photosystem II core preparation, active only in DPC[†] to DCIP electron transport, was prepared essentially according to Bricker *et al.*[16] A photosystem II core preparation capable of oxygen evolution was prepared according to Ikeuchi *et al.*[2] During the preparation, protease inhibitors were used as described in Ref. 17. Triton X-114/water phase-partitioning[18] was performed as in Ref. 10. Sodium dodecylsulfate-polyacrylamide gel electrophoresis was run at 0 °C in the buffer system of Laemmli,[19] in the presence of 4 M urea, using a 12–22.5 % acrylamide gradient. The 5 kDa hydrophilic polypeptide was prepared by ion-exchange chromatography as described previously.[10] Cytochrome *b*-559 was purified as described by Metz *et al.*[20]

Results and discussion

A large number of thylakoid polypeptides with molecular weights lower than the 9 kDa apopoly-

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†DCIP = 2,6-dichlorophenolindophenol; DPC = 1,5-diphenylcarbazide.

peptide of cytochrome *b*-559 could be resolved with the sodium dodecylsulfate urea-polyacrylamide gel electrophoresis system employed. The levels of some polypeptides were increased in the stroma lamellae, whereas those of others were increased in a photosystem II membrane preparation; this indicated that some of these low molecular weight polypeptides were photosystem II constituents. Two photosystem II core preparations, one active only in DPC to DCIP electron transport, the other active also in oxygen evolution, were therefore analyzed with this high resolution gel electrophoresis system.

In accordance with previous studies,[1-3,16,21] the photosystem II core preparations were found to contain the apopolypeptides of CPa_I and CPa_{II} of 47 and 43 kDa, respectively, the Q_B and D2 apopolypeptides of approximately 32 kDa, and the 9 kDa apopolypeptide of cyt *b*-559. In addition, the oxygen-evolving core preparation contained the extrinsic 33 kDa polypeptide. Strikingly, both preparations gave rise to prominent bands in the low molecular weight region: the photosystem II core preparation capable of oxygen evolution contained five low molecular weight constituents, whereas the photosystem II core preparation which was *not* capable of oxygen evolution contained four (Fig. 1). Both preparations contained polypeptides of molecular weight 4, 5 and 5.5 kDa. Additionally, the oxygen-evolving core preparation contained two polypeptides of 6.5 and 7 kDa while the other core preparation contained a 6.8 kDa polypeptide.

To examine whether the low molecular weight polypeptides present in the oxygen-evolving photosystem II core preparation were true components of the photosystem II core, we measured their co-purification with known photosystem II core constituents. Co-purification was determined as the relative abundance of the polypeptides in the photosystem II core complex compared to the starting material (the photosystem II membranes). The value was normalized to 100 % for the known photosystem II core components, i.e. the CPa_I, CPa_{II}, Q_B and D2 apopolypeptides. The five low molecular weight polypeptides all showed a quite high degree of co-purification, ranging from 45 % to 148 %. The 33 kDa extrinsic protein and the cytochrome *b*-559 co-purified to 99 % and 69 %, respectively. In contrast, all polypeptides of molecular weight between 9 and 32 kDa, including the extrinsic 16 kDa and 23 kDa proteins and the apopolypeptides of LHC-II, showed low degrees of co-purification. Thus, the low molecular weight polypeptides of the oxygen-evolving photosystem II core preparation showed a quite stringent co-purification with the four larger and previously characterized constituents of the photosystem II core complex.

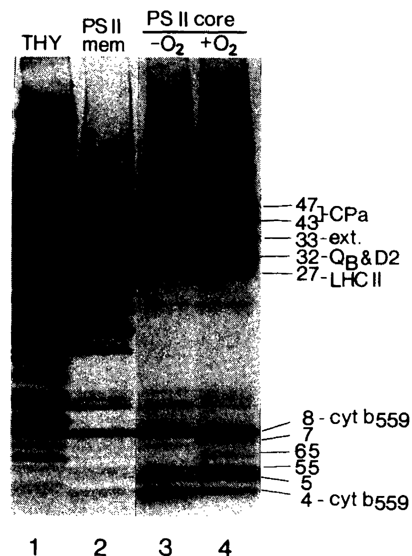

Fig. 1. Sodium dodecylsulfate-polyacrylamide gel electrophoresis of: (1) thylakoid membranes, (2) photosystem II membranes, (3) photosystem II core preparation according to Bricker *et al.* and (4) photosystem II core preparation according to Ikeuchi *et al.*

The possibility that the low molecular weight polypeptides were products of proteolytic breakdown was examined by performing the preparation of the photosystem II core in the presence of protease inhibitors. However, these did not alter either the identity or the abundance of the low molecular weight polypeptides, and it is therefore improbable that they are products of proteolytic breakdown.

Triton X-114/water phase-partitioning, a technique which separates hydrophilic polypeptides from hydrophobic, was employed to examine the nature of the low molecular weight polypeptides of the photosystem II core. All but one of these partitioned into the hydrophobic Triton phase, only one of 5 kDa being found in the aqueous phase. The latter 5 kDa hydrophilic polypeptide

was later isolated by ion-exchange chromatography and characterized.[10] After isolation it contained no metals or cofactors exhibiting absorption in the visible region of the spectrum. The 4 kDa hydrophobic polypeptide was shown to be the newly discovered 4.3 kDa subunit of cytochrome *b*-559.[11] This analysis was performed by co-electrophoresis of the purified cytochrome (not shown).

In conclusion, we regard these low molecular weight polypeptides as constituents of the photosystem II core. This is supported by the finding that a newly developed photosystem II core preparation also contains the 4, 5 and 5.5 kDa polypeptides that are present in both of the previously described photosystem II core preparations.[22] In addition, this preparation contained the 6.8 kDa polypeptide mentioned earlier. The 4, 5 and 5.5 kDa polypeptides present in all these preparations may be involved in the primary photochemistry of photosystem II, whereas it seems unlikely that the 6.5, 6.8 and 7 kDa polypeptides are involved in these reactions.

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