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Characterization of low molecular mass proteins of photosystem II by N-terminal sequencing

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The N-terminal amino acid sequence of four chloroplast proteins with the apparent molecular masses of 3.7, 6.5, 7 and 8 kDa has been determined. Two of the proteins (6.5 and 7 kDa) turned out to have the same amino acid sequence. This sequence, and the sequence of the 3.7 kDa protein, did not correspond to any of the known chloroplast protein sequences, or to any of the open reading frames of the chloroplast genome. The 8 kDa protein, which could be distinguished from the cytochrome *b*-559 band only at increased urea concentrations, was partially purified and found to be a degradation product of the extrinsic 16 kDa protein.

Photosystem II protein; N-terminal sequencing; (Spinach)

1. INTRODUCTION

It has recently been shown that several types of photosystem II core preparations contain proteins with molecular masses lower than the 9 kDa subunit of cytochrome *b*-559 [1]. The identity and role of these proteins are still mainly unknown. Besides a possible functional role in photosystem II, they may represent transit sequences of larger proteins or other proteolytic products.

In the present study four, of the so far not studied, low molecular mass proteins were sequenced.

2. MATERIALS AND METHODS

Photosystem II particles were prepared according to the detergent method in [2].

SDS-gel electrophoresis with 4 M urea and a 10–20% polyacrylamide gradient or with 8 M urea and a 13–26% stained with Coomassie brilliant blue R-250 or silver stained according to Guevara et al. [4].

Polyvinylidene difluoride (PVDF) membranes were obtained from Millipore. The proteins were transferred to the PVDF membrane using a JKA-Biotech semidry electroblotter essentially as in [5] except that the gels were not soaked in transfer buffer.

The protein bands were cut out and sequenced on an Applied Biosystem model 470 sequenator [5].

To purify the 8 kDa protein, photosystem II particles were solubilized with a mixture of 2% Zwittergent TM314 and 2% digitonin in 10 mM Mes, pH 6.0. The solubilized material was then loaded on an HPLC anion-exchange column (TSK DEAE-3SW, LKB), preequilibrated with 10 mM Mes, pH 6.0, and 0.1% Zwittergent TM 314. The bound material was then eluted with an NaCl gradient from 0 to 1 M, in 10 mM Mes, pH 6.0, and 0.1% Zwittergent TM314.

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Abbreviation: Mes, 2(*N*-Morpholino)ethanesulfonic acid

3. RESULTS AND DISCUSSION

As shown in fig.1, several low molecular mass proteins can be seen when photosystem II particles are subjected to SDS-PAGE in the presence of 4 M urea at low temperature (0°C). Similar results have earlier been obtained for different photosystem II core preparations [1]. Some of the low molecular mass proteins have already been identified or partly characterized. Thus, the 10 kDa protein is in-

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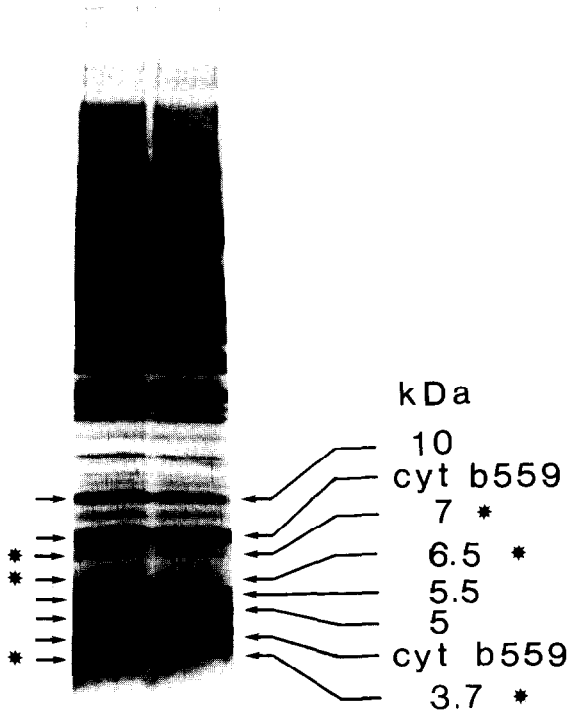

Fig.1. Polypeptide pattern of photosystem II particles (silver stained). The protein bands labeled with stars were sequenced.

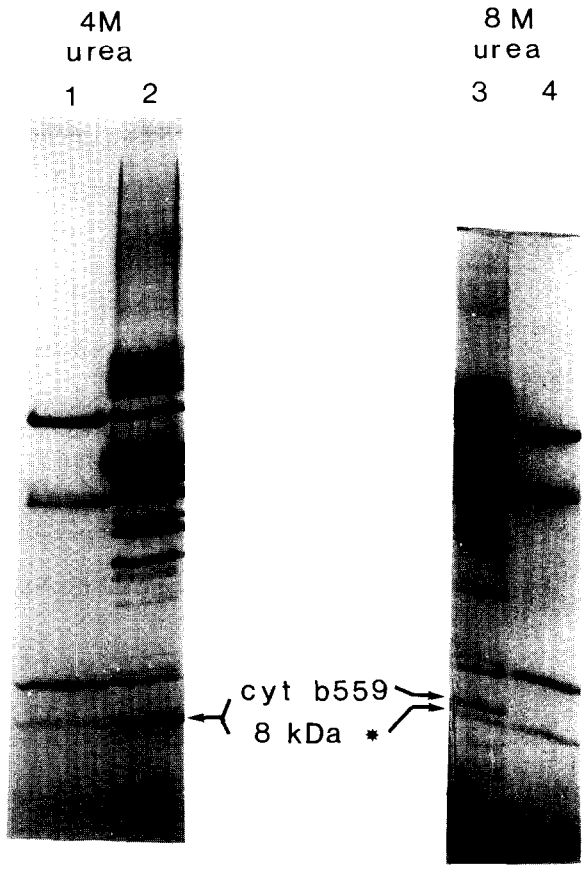

Fig.2. Polypeptide pattern of photosystem II particles (lane 2 and 3) and the partially purified 8 kDa protein (lane 1 and 4). Electrophoresis was run in the presence of 4 M urea (lane 1 and 2) or 8 M urea (lane 3 and 4), and the gels were stained with Coomassie brilliant blue.

volved in binding of the 23 kDA hydrophilic protein [6], the 8 kDa and 4 kDa proteins are subunits of cytochrome *b*-559 [7] and the 5 kDa protein is a hydrophilic protein isolated earlier [1]. There is also a phosphoprotein in the 8–10 kDa region [8]. The remaining protein bands in the low molecular mass region stain only weakly, which can be due to their small size. However, the amounts may still be significant for a function in photosystem II. In an attempt to improve the resolution of the gel system, 8 M urea was used instead of 4 M.

As shown in fig.2, the sharpness of the protein bands of photosystem II particles was not significantly improved, but a previously undetected low molecular mass band at approx. 8 kDa appeared. Coelectrophoresis of the protein, partially purified by ion-exchange HPLC (fig.3), revealed that this 8 kDa protein comigrated with the large subunit of cytochrome *b*-59 in the system with 4 M urea (fig.2). In order to characterize further the low molecular mass proteins, those marked in fig.1 and the new protein band which apperared in 8 M urea (fig.2) were electroblotted over to a PVDF membrane and then cut out and N-terminally sequenced.

Three of the obtained sequences differed from each other, while the 6.5 kDa protein showed the same N-terminal sequence as the 7 kDa protein (fig.4). The 6.5 kDa protein is therefore considered to be a degradation product or conformational isomer of the 7 kDa protein. Interestingly another weaker background sequence was found in the 3.7 kDa protein band, which may correspond to the 3.2 kDa protein found in [1]. No homology was found with other plant proteins, even if the sequenced proteins were tested in steps of only 4 amino acids. This comparison suggested that the 7

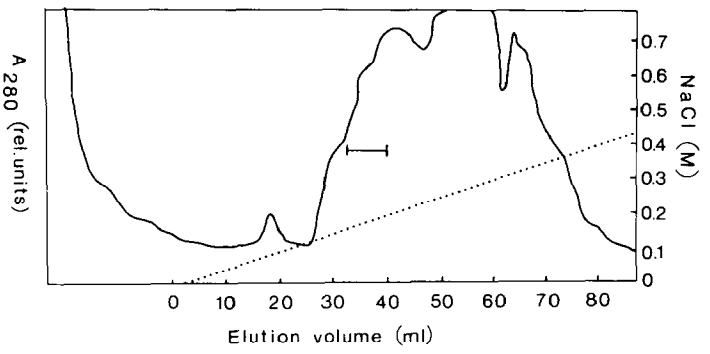

Fig.3. Elution diagram of ion-exchange HPLC of detergent solubilized photosystem II particles. The bar shows the region where the 8 kDa protein was found.

amino acid	protein band (kDa)				
nr	8	7	6.5	3.7	(3.2)
1	?	Leu	Leu	?	?
	Ala	Val	Val	Leu	Glu
	Arg	Asp	Asp	Pro	?
	Pro	Glu	Glu	Glu	Ala
5	Ile	Arg	?	Ala	?
	Val	Met	Met	Tyr	Ser
	Val	Ser	Ser	Ala	Lev
	Gly	Thr	Thr	Phe	?
	Pro	Glu	Glu	Leu	Asn
10	Pro	Gly	Gly	Ser	
	Pro	Thr		Pro	
	Pro	Gly		Ile	
	Leu				
	Ser				
15	Gly				
	Gly				
	Leu				
	Pro				
	Gly				
20	Thr				
	?				
	Asn				
	Ser				
	Asp				

Fig.4. N-terminal partial sequence of the four proteins marked with stars in figs 1 and 2.

and 3.7 kDa proteins, and the background sequence probably originating from the 3.2 kDa protein, are unique. Furthermore they are probably encoded by the nuclear genome as their partial sequences do not correspond to any of the open reading frames of the chloroplast genome [9]. As was shown earlier, at least the 7 kDa protein is present in several, if not all, different oxygen-evolving photosystem II core preparations and may therefore be important for the photosystem II function.

The N-terminal sequence of the 8 kDa protein (fig.4) is identical to that reported for the hydrophilic 16 kDa protein of the oxygen-evolving system [10]. The 8 kDa protein thus appears to be a degradation product, in addition to the C-terminal fragment found by Kuwabara et al. [11]. Notably, antibodies raised against the purified 16 kDa protein did not react with the 8 kDa protein but only with the 16 kDa protein and the 'Kuwabara fragment' (not shown). Salt wash experiments indicate that the 8 kDa protein binds stronger to the membrane than the 16 kDa protein itself (not shown). This suggests that the 8 kDa fragment carries the part of the 16 kDa protein that is responsible for binding to the membrane component. This is in agreement with the results of Kuwabara et al. [11], who found that proteolytic removal of a small N-terminal part of the 16 kDa protein resulted in a loss of ability to bind and reconstitute oxygen evolution.

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