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A 10 kDa polypeptide associated with the oxygen-evolving complex of photosystem II has a putative C-terminal non-cleavable thylakoid transfer domain

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The N-terminal amino acid sequence of the 10 kDa polypeptide associated with the oxygen-evolving complex of wheat photosystem II has been determined and shown to be homologous to the amino acid sequence of the product of the ST-LS1 gene from potato. The N-terminal sequence of the mature protein indicates that the polypeptide is synthesized with a 39 amino acid N-terminal presequence which is similar to chloroplast import sequences but which lacks a hydrophobic domain for transfer of the protein across the thylakoid membrane. The mature polypeptide has a C-terminal hydrophobic region which shows homology to the hydrophobic thylakoid transfer domain of other lumenal proteins and this hydrophobic region of the 10 kDa polypeptide is suggested to facilitate transfer of the protein across the thylakoid membrane.

Chloroplast; Photosystem II; Oxygen-evolving complex; Presequence; Thylakoid transfer domain; (Wheat)

1. INTRODUCTION

Reactions occurring on the oxidising side of photosystem II require the participation of a group of hydrophilic proteins associated with the lumenal side of the thylakoid membrane [1]. The best characterised of these proteins are the 33, 23 and 16 kDa polypeptides released from photosystem II preparations by alkaline Tris washing [2]. All three proteins are known to be encoded by genes located in the nucleus [3]. The amino acid sequences of the 33, 23 and 16 kDa polypeptides, derived from the corresponding cDNA nucleotide sequences, reveal that they are synthesized in the cytoplasm with N-terminal amino acid extensions [4,5]. The N-terminal presequences of these proteins contain regions required for targeting to, and transfer across, the chloroplast envelope membranes, similar to other chloroplast proteins [6]. They also contain an additional hydrophobic region, immediately preceding the final processing site, which is thought to be required for the transfer of the protein across the thylakoid membrane and into the lumen. This hydrophobic thylakoid transfer domain is also present in the presequence of plastocyanin [7], another hydrophilic protein located in the thylakoid lumen.

Information on the presequences of other proteins located in the thylakoid lumen would be useful for further comparison of the amino acid sequence domains required for the transfer of proteins across the thylakoid membrane. Photosystem II contains a number of other polypeptides which may be associated with the oxygen-evolving complex [8], and are located in the thylakoid lumen [9]. A polypeptide of 10 kDa has been shown to be associated with the 33 kDa and 23 kDa polypeptides by immunoprecipitation of detergent-solubilised photosystem II preparations [8]. This 10 kDa polypeptide can be extracted from inside-

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Abbreviations: PVDF, polyvinylidene difluoride; PTH, phenylthiohydantoin

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out thylakoid vesicles by washing with alkaline Tris solutions [9], indicating that it is an extrinsic protein located in the thylakoid lumen. The amino acid composition of the 10 kDa polypeptide has been reported [10], and indicates that it is a hydrophobic protein. In this paper we report that the N-terminal amino acid sequence of the 10 kDa polypeptide of wheat photosystem II corresponds to the derived amino acid sequence of a light-inducible gene, ST-LS1, from potato [11]. This has allowed us to compare the presequence of the 10 kDa polypeptide with that of other proteins located in the thylakoid lumen and indicates that the preprotein does not contain a cleavable N-terminal hydrophobic thylakoid transfer domain. Instead, the mature protein contains a C-terminal hydrophobic region which is similar to the thylakoid transfer domains of other lumenal proteins and which, we suggest, acts as a non-cleavable thylakoid transfer domain.

2. MATERIALS AND METHODS

Photosystem II was purified from washed thylakoid membranes isolated from 10-day-old wheat leaves using the method of Berthold et al. [12]. Photosystem II polypeptides were fractionated by SDS-gel electrophoresis by the method of Fling and Gregerson [13] except the resolving gel contained 18% acrylamide and 5 M urea. For Western blotting, polypeptides were transferred to nitrocellulose membranes [14] and probed with antisera using the procedure of Yen and Webster [15]. For amino acid sequencing, gels were soaked in transfer buffer (20 mM Tris, 150 mM glycine, 20% methanol and 0.05% SDS) before electroblotting to PVDF membrane at 250 mA for 2 h in transfer buffer [14,16]. Amino acid sequence analysis was performed on an Applied Biosystems 470A gas-phase sequencer coupled to a 120A PTH-analyser, all under the control of a 900A data module. Samples of PVDF membrane bearing electroblotted 10 kDa protein were cut into 5–10 mm lengths, each of which was 'frayed' with a scalpel blade into joined strips of 0.5–1 mm width to allow free flow of reagent in the sample cartridge of the sequencer. The PVDF membrane strips were held in place in the upper cartridge block with a TFA-treated sample disc (without biobrene plus) and subjected to sequence analysis using RUN-1 software. The 10 kDa polypeptide gave an initial yield of 18 pmol and an average repetitive yield of 91%; the sequence could not be read unambiguously after Ala-24 because of a sudden drop in yield after this residue.

3. RESULTS AND DISCUSSION

Alkaline Tris washing of wheat photosystem II preparations removed a polypeptide of 10 kDa (fig.1). The solubility of the protein in alkaline Tris

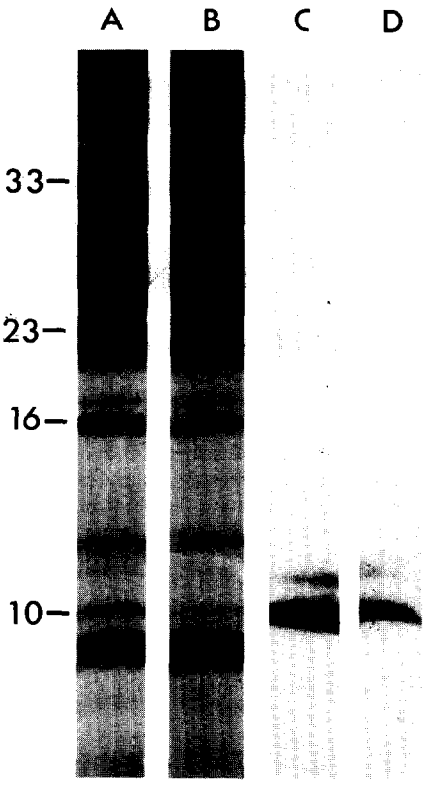

Fig.1. Polypeptide profile of wheat photosystem II preparations (20 μg chlorophyll; A) and Tris-washed photosystem II preparations (B, C and D). Autoradiographs of Western blots of A and B probed with antisera against the 10 kDa polypeptide of the oxygen-evolving complex.

and its molecular mass estimated by SDS-PAGE suggested that it corresponds to the 10 kDa polypeptide associated with the oxygen-evolving complex characterised from spinach photosystem II preparations [9]. Antiserum raised against the spinach 10 kDa polypeptide cross-reacted with this wheat 10 kDa polypeptide (fig.1), thus confirming its identity. Following transfer to PVDF membrane, the N-terminal amino acid sequence of the wheat 10 kDa polypeptide was determined by gas-phase sequencing. The sequence of the first 24 amino acids of the N-terminal region of the 10 kDa protein (fig.2) shows considerable homology to a region (Ser-40–Ser-65) of the product of ST-LS1, a light-inducible nuclear gene from potato [11] (fig.2). The homology indicates that ST-LS1 encodes the 10 kDa polypeptide associated with the oxygen-evolving complex of

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                                                        15
ST-LS1   M A S T V M S S L S L K P T F
                                                        30
         T L E K T S V K G L P S L A R
                                                        45
         S S S S F K V V A S G V K K L
10kDa                      S G G K K I
                                                        60
         K T D K P Y G I N G S M A L R
         K V D K P L G L G G G L T V -
                                                        75
         D G V D A S C R K P K G K G V
         D - I D A
                                                        90
         Y Q Y V D K Y G A N V D G Y S
                                                       105
         P I Y N T D E W S P S G D V Y
                                                       120
         V G G T T G L A I W A V T L V
                                                       135
         G L L A G G A L L V Y N T S A

         L A Q.
```

Fig.2. Comparison of the amino acid sequence of ST-LS1 [11] with the sequence of the first 24 amino acids determined from the 10 kDa wheat polypeptide. Identical residues are boxed. Gaps have been introduced into the amino acid sequence of the mature wheat polypeptide to give maximum alignment.

photosystem II. The N-terminal amino acid of the mature ST-LS1 protein is therefore identified as Ser-40. This indicates that the mature protein is 99 amino acid residues in length and is synthesized in the cytoplasm with an N-terminal presequence of 39 amino acid residues. A comparison of the amino acid composition of the mature ST-LS1 protein, deduced from the nucleotide sequence, with the amino acid composition of the spinach 10 kDa polypeptide [10], using the analysis of Cornish-Bowden [17], provides further evidence that ST-LS1 encodes the 10 kDa polypeptide.

Studies using inside-out thylakoid vesicles have conclusively shown that the 10 kDa polypeptide is located on the lumenal side of the thylakoid membrane [8]. Thus, the N-terminal extension might be expected to contain the information required for transfer of the protein across the chloroplast envelope and thylakoid membranes on route to the lumen. However, the presequence of 39 amino acid residues is considerably shorter than for other lumenal proteins (65–84 residues) and lacks the large hydrophobic domain immediately prior to the final processing site. This hydrophobic domain has been shown to be required for transfer of plastocyanin across the thylakoid membrane [7] and is suggested to play a similar role in the transfer of the 33 kDa, 23 kDa and 16 kDa polypeptides into the thylakoid lumen [4,5]. The only extended hydrophobic region in the protein deduced from the potato ST-LS1 gene is located near the C-terminus (fig.3). We have compared this deduced amino acid sequence with the N-terminal presequences of other lumenal proteins, using a computer match program. This indicates there is homology between the hydrophobic thylakoid transfer domains of the lumenal proteins and the hydrophobic region at the C-terminus of the 10 kDa polypeptide (fig.4). Particularly striking is the sequence, Ala-Leu-Ala, which forms the last three amino acids of the thylakoid transfer sequence of pea plastocyanin (Last and Gray, unpublished) and is also present at the C-terminus of the 10 kDa polypeptide. A similar sequence, Ala-X-Ala, is also present in the thylakoid transfer domains of the 33 kDa and 23 kDa polypeptides of the water-oxidising complex [4,5]. This sequence probably acts as part of the recognition sequence of the lumenal processing protease, and similar sequences are found at the cleavage site for signal peptidase in animal and bacterial signal peptides [19].

We suggest that the hydrophobic C-terminal region of the 10 kDa polypeptide functions as the thylakoid transfer domain for the import of the polypeptide into the thylakoid lumen. The N-terminal presequence is suggested to function solely as a chloroplast import sequence for transfer across the chloroplast envelope and is processed by a stromal protease prior to transfer of the mature protein across the thylakoid membrane. The

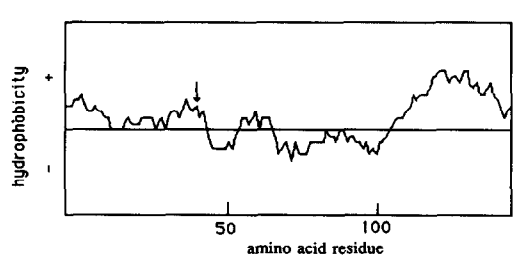

Fig.3. Hydrophobicity plot of ST-LS1 generated according to Kyte and Doolittle [18] using an amino acid window of 11. The predicted processing site is indicated by the arrow.

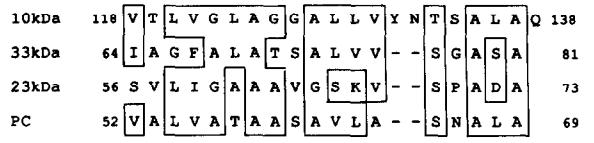

Fig.4. Alignment of the C-terminal region of the mature 10 kDa polypeptide with the thylakoid transfer domains in the presequences of the 33 kDa and 23 kDa polypeptides of the oxygen-evolving complex from pea (Wales, R., unpublished) and pea plastocyanin (Last, D.I., unpublished). Identical residues and conservative replacements are boxed.

stromal processing site is most probably the peptide bond between Ala-39 and Ser-40 immediately following a region predicted to form a β-sheet secondary structure. Similar secondary structures are observed within the presequences of other proteins located within the thylakoid lumen [4–6]. A basic residue (Lys-36) just before the processing site is also commonly found in the vicinity of the stromal processing site of other chloroplast proteins [6]. The predicted secondary structure homology may be required for recognition by the stromal processing protease. Removal of the N-terminal presequence by the stromal processing protease is suggested to release the mature 10 kDa polypeptide which is targeted to the thylakoid lumen by its C-terminal hydrophobic region. The molecular mass of the mature 10 kDa polypeptide determined by SDS-gel electrophoresis is close to that predicted from the deduced amino acid sequence suggesting that the C-terminal hydrophobic region of the protein is not removed. However, if the sequence Ala-Leu-Ala is recognised by the lumenal processing protease then the C-terminal glutamine residue (Gln-138) may be removed. The C-terminal region (residues 104–138) of the 10 kDa polypeptide is presumably responsible for the hydrophobic nature of the mature protein, as indicated by partitioning into Triton X-114 [10]. However, the fact that the protein is washed off inside-out thylakoid vesicles by alkaline Tris, in the complete absence of detergent [8], indicates that the protein is not an intrinsic component of the thylakoid membrane. The amino acid sequence of the protein shows no homology to other proteins involved in metal binding and provides no obvious indications as to its role in photosystem II.

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