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A fifth chloroplast-encoded polypeptide is present in the photosystem II reaction centre complex

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The small polypeptides present in the pea photosystem II reaction centre complex have been separated by high-resolution SDS-polyacrylamide gel electrophoresis and characterised by N-terminal amino acid sequencing. Two polypeptides are identified as the α- and β-subunits of cytochrome *b*-559 which are the products of the *psb*E and *psb*F genes. A third polypeptide, of 4.5 kDa, is identified as the product of a small open reading frame in chloroplast DNA. The gene for this polypeptide, *psb*I, is located just downstream of the *psb*K gene in chloroplast DNA.

Photosystem II reaction center; Cytochrome *b*-559; Gene, *psb*E; Gene, *psb*F; Gene, *psb*I; (Pea)

1. INTRODUCTION

Photosystem II carries out the light-driven transfer of electrons from water to plastoquinone in the photosynthetic membranes of plants and cyanobacteria. Our understanding of the structure and mechanism of action of photosystem II has been improved recently with the isolation of a reaction centre complex [1,2] showing many similarities to the reaction centre of photosynthetic purple bacteria [3,4]. The reaction centre complex contains the primary electron donor P680, phaeophytin *a* and chlorophyll *a* associated with the two polypeptides known as D1 and D2, which show considerable sequence homology with the L and M subunits of the bacterial reaction centre [5,6]. In addition the reaction centre complex contains cytochrome *b*-559 which is associated with a polypeptide of 9 kDa [1]. A small polypeptide of 4 kDa is also associated with the complex and has been ascribed to cytochrome *b*-559 [1,2] but the polypeptide has not been characterised further. We have separated the small polypeptides of the pea photosystem II reaction centre complex by high resolution SDS-polyacrylamide gel electrophoresis and have characterised the polypeptides by N-terminal amino acid sequencing. This has confirmed the presence of both polypeptides of cytochrome *b*-559 in the reaction centre complex and has identified a fifth polypeptide component of the complex. The determined N-terminal amino acid sequence of a 4.5 kDa polypeptide matches the product of a small open reading frame in chloroplast DNA from several plants.

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2. MATERIALS AND METHODS

2.1. *Purification of the photosystem II reaction centre*

Pea photosystem II membrane preparations [7] solubilised in 50 mM Tris-HCl, pH 7.2, containing 4% Triton X-100 were applied to a DEAE-Fractogel (Merck) column and, following extensive washing with 50 mM Tris-HCl, pH 7.2, containing 20 mM NaCl and 0.2% Triton X-100, the bound material was eluted with a gradient of increasing NaCl concentration [2]. Fractions (1 ml) from the gradient containing the photosystem II reaction centre were collected and samples were subjected to high resolution SDS-polyacrylamide gel electrophoresis on 15–22% gels using buffers containing twice the normal Tris concentration [8]. Photosystem II reaction centre-containing

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fractions were pooled and purified by a second chromatographic step on DEAE-Fractogel.

2.2. *N-terminal amino acid sequencing*

The reaction centre polypeptides, separated by SDS-polyacrylamide gel electrophoresis, were electroblotted to polyvinylidene difluoride (PVDF) membrane (Millipore) and visualised by staining with Coomassie blue in 50% methanol for 5 min followed by destaining in 50% methanol and 10% acetic acid [9]. The immobilised polypeptides were submitted to sequence analysis in an Applied Biosystems 477/120 pulsed liquid protein sequencer. Samples which failed to give sequence information were removed from the sequencer together with the polybrene-coated glass fibre disc and incubated overnight at room temperature in the dark with 30 μl of CNBr (100 mg/ml) in 70% formic acid applied to the glass fibre disc in a 1.5 ml Eppendorf tube flushed with argon. The acid and CNBr were removed under vacuum over NaOH and the glass fibre disc resubmitted to sequence analysis.

3. RESULTS AND DISCUSSION

High resolution SDS-polyacrylamide gel electrophoresis of preparations of the photosystem II reaction centre complex from pea chloroplast membranes revealed the presence of three small polypeptides, in addition to the D1 and D2 polypeptides and the slower migrating D1–D2 dimers (fig.1A). By reference to the mobility of cyanogen bromide-cleavage products of myoglobin, the molecular masses of the three small polypeptides were estimated to be 6.8, 4.5 and 2.0 kDa. However, it must be pointed out that estimates of the molecular mass of small polypeptides obtained by this high resolution electrophoresis system are not particularly reliable. All three polypeptides copurified with the D1 and D2 polypeptides during elution from DEAE-Fractogel (fig.1A). This chromatography step separates the reaction centre complex from the other components of the photosystem II complex, including the light-harvesting chlorophyll *a*/*b*-binding proteins (LHCII) and the 47 and 44 kDa chlorophyll *a*-binding polypeptides of the antenna complex. The copurification of the small polypeptides with the D1–D2 polypeptides clearly suggests that the small polypeptides are part of the reaction centre complex.

The small polypeptides separated by SDS-polyacrylamide gel electrophoresis were transferred to PVDF membrane by electroblotting (fig.1B) and the individual polypeptides were subjected to N-terminal amino acid sequencing. The 6.8 kDa polypeptide gave a sequence that was identical to the product of the pea chloroplast *psb*E gene (Willey, D.L. et al., unpublished) except for the removal of the initiating methionine residue. The *psb*E gene has been shown to encode a cytochrome *b*-559 polypeptide migrating at 9 kDa in other gel systems [10,11]. The 4.5 and 2.0 kDa polypeptides failed to give sequence information presumably due to N-terminal blockage but sequence analysis after treatment with cyanogen bromide was successful. The polypeptides on the PVDF membrane were treated with cyanogen bromide to cleave at methionine residues and the polypeptides were resequenced. The 2.0 kDa polypeptide gave a mixture of three residues at each cycle. The sequences could be recognised as derived from the *psb*F gene product which had been cleaved after Met-1, Met-34 and Trp-14 (fig.2C). Cleavage after tryptophan is not unusual when high concentrations of cyanogen bromide are employed [12]. The *psb*F gene has been identified as the gene for the small polypeptide of cytochrome *b*-559, which migrates at 4 kDa in other gel systems [10,13]. The N-terminal sequencing therefore establishes the presence of both polypeptides of cytochrome *b*-559 in the photosystem II reaction centre complex.

The 4.5 kDa polypeptide yielded a single sequence after cleavage with cyanogen bromide which matched that derived from a small open reading frame in the chloroplast genomes of tobacco [14], liverwort [15] and wheat [16] (fig.2B). The incubation conditions during cyanogen bromide treatment have clearly removed the unidentified N-terminal block while failing to cleave after Met-1. The open reading frame encodes a protein of 36 amino acid residues, of which residues 6–26 are hydrophobic and may constitute a membrane-spanning region. A single charged residue (Lys-5) is present at the N-terminus of the tobacco, wheat and liverwort polypeptides, but proline was detected in this position in pea. The C-terminal region is highly charged with basic and acidic residues. The polypeptide is therefore similar in many respects to the first 40 residues of the H subunit of the bacterial reaction centre.

The open reading frame encoding the 4.5 kDa polypeptide has been called *psb*I in maize, based on the detection of a polypeptide in photosystem II preparations using antibodies raised against a synthetic peptide deduced from the sequence of the

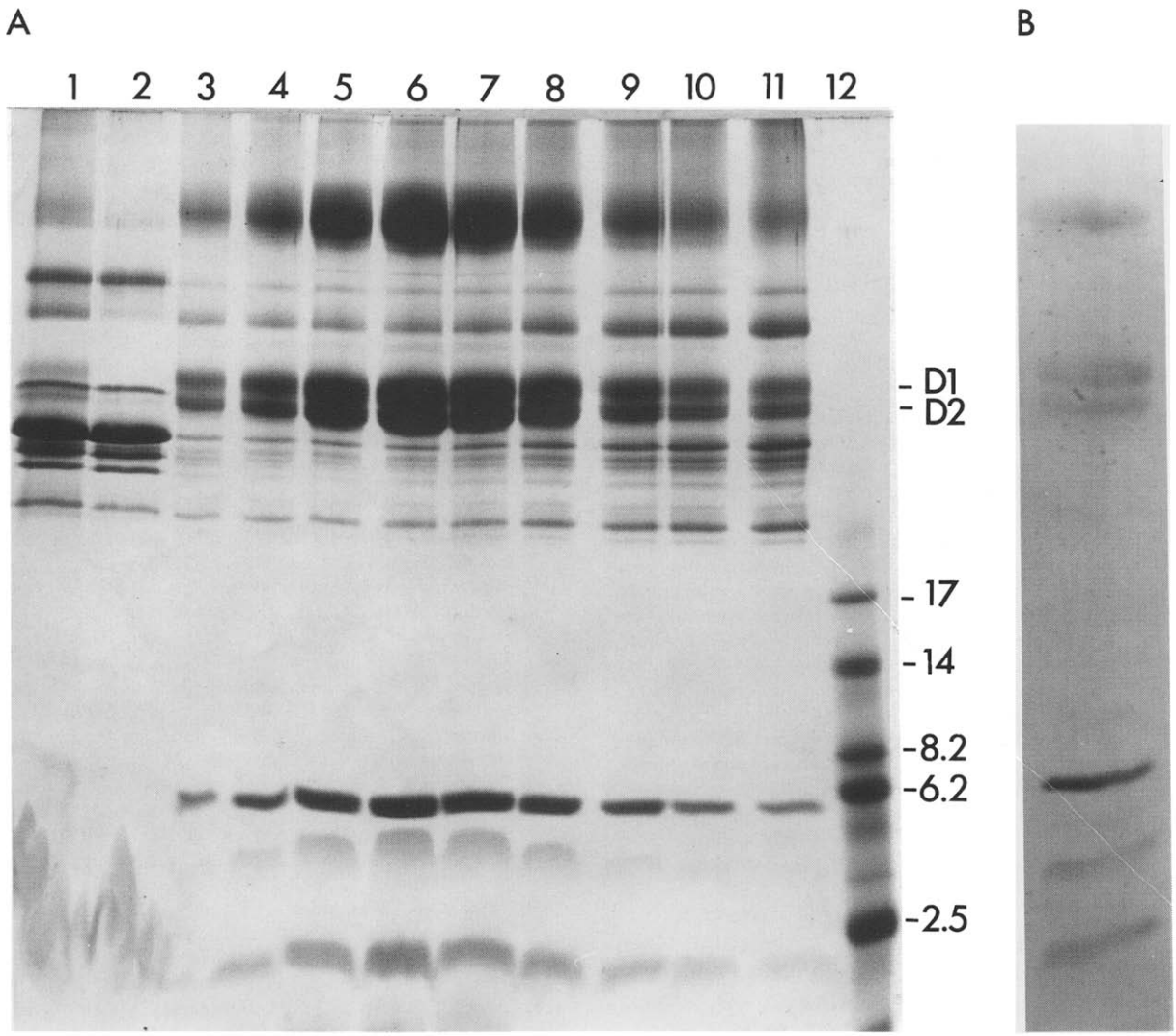

Fig.1. Polypeptides of the pea photosystem II reaction centre core complex. (A) High resolution SDS-polyacrylamide gel electrophoresis of fractions eluted from a DEAE-Factogel column. Lanes: 1, photosystem II preparation applied to column; 2, unbound material; 3–11, fractions containing the photosystem II reaction centre; 12, molecular mass markers of cyanogen bromide-cleaved myoglobin. (B) Photosystem II reaction centre polypeptides immobilised on PVDF membrane. The transfer was optimised for the small polypeptides; transfer of the D1 and D2 polypeptides was incomplete.

open reading frame [17]. This nomenclature has also been used for a homologous open reading frame in tobacco chloroplast DNA [18]. The *psb*I gene is located just downstream from the recently described *psb*K gene which also encodes a small polypeptide associated with photosystem II preparations [19]. Two small open reading frames homologous to those in the tobacco chloroplast genome are also present in the liverwort chloroplast genome [15]. It seems likely that the *psb*I and *psb*K genes are cotranscribed in tobacco [19]. In wheat the *psb*I gene is located approximately 1.2 kbp upstream from the *psb*D gene for the D2 polypeptide [16] and may be cotranscribed with the *psb*D and *psb*C genes (Hird, S.M. et al., unpublished).

These studies have shown that the photosystem II reaction centre complex from pea consists of

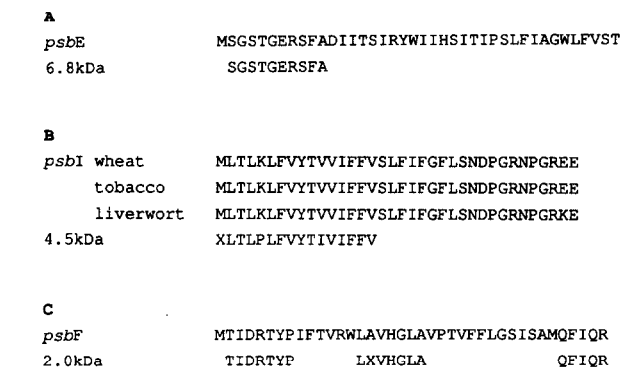

Fig.2. N-terminal amino acid sequences of the 6.8, 4.5, and 2.0 kDa polypeptides of the photosystem II reaction centre. (A) The N-terminal amino acid sequence of the 6.8 kDa polypeptide compared to residues 1–40 of the product predicted for the pea *psb*E gene (Willey, D.L., unpublished) encoding the α-subunit of cytochrome *b*-559. Sequence was obtained with an initial yield of 150 pmol. (B) The N-terminal amino acid sequence of the 4.5 kDa polypeptide compared to the amino acid sequence derived from an open reading frame in the chloroplast genomes of tobacco [14], liverwort [15] and wheat [16]. Sequence was obtained with an initial yield of 17 pmol. (C) Amino acid sequence analysis of the cyanogen bromide-cleaved 2.0 kDa polypeptide. Three residues were obtained at each cycle and are matched to the predicted product of the pea *psb*F gene (Willey, D.L., unpublished) encoding the β-subunit of cytochrome *b*-559. Sequence was obtained with initial yields of 2, 4, and 7 pmol for the three peptides starting with Thr-2, Leu-15 and Gln-35, respectively.

five polypeptides, all encoded in the chloroplast genome. The D1 and D2 polypeptides are the location of the redox components Z, D, P680, phaeophytin *a*, Q_A and Q_B [1,2,20], and are encoded in the chloroplast genes *psb*A and *psb*D [21,22]. The 6.8 and 2.0 kDa products of the *psb*E and *psb*F genes are the location of the haem group of cytochrome *b*-559. However, the role of the 4.5 kDa product of the *psb*I gene is not clear. The possibility that the 4.5 kDa polypeptide fulfills a similar function to the H subunit of the bacterial reaction centre requires further investigation.

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