A photosystem II polypeptide is encoded by an open reading frame co-transcribed with genes for cytochrome b-559 in wheat chloroplast DNA

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Received 2 August 1988; accepted in revised form 19 October 1988

Key words: chloroplast DNA, cytochrome b-559, photosystem II, psbL, wheat

Abstract

The N-terminal amino acid sequence of a 3.2 kDa photosystem II polypeptide is shown to be identical to that of a polypeptide encoded by an open reading frame of 38 codons (orf38) in wheat chloroplast DNA. Orf38 is located just downstream of the psbE and psbF genes for the polypeptides of cytochrome b-559. Analysis of the transcription of this region of chloroplast DNA shows that psbE, psbF and orf38 are co-transcribed to give a 1.1 kb polycistronic transcript which also contains another open reading frame of 40 codons. The orf38 and orf40 products are hydrophobic polypeptides which are both predicted to span the thylakoid membrane once. Orf38 and orf40 are highly conserved, and map to similar locations adjacent to psbE and psbF, in all organisms from which this region of DNA has been sequenced. We propose that orf38 is named psbL.

Introduction

Photosystem II is a multi-protein complex located in the thylakoid membrane utilising captured light energy for the oxidation of water and the reduction of plastoquinone. Photosystem II contains approximately 20 different polypeptides which are encoded by genes distributed between the nuclear and the chloroplast genomes. The chlorophyll a/b-binding polypeptides of the light-harvesting complex (LHCII) and the hydrophilic polypeptides of the oxygen-evolving complex are nuclear-encoded whereas the polypeptides of the photosystem II core complex are all encoded by genes located in the chloroplast (for review see [12]). The identified chloroplast genes encode the 47 kDa (psbB) and 43 kDa (psbC) chlorophyll-binding proteins, the D1 (psbA) and D2 (psbD) polypeptides which bind the chlorophyll, phaeophytin and plastoquinone molecules of the reaction centre and the 9 kDa (psbE) and 4 kDa (psbF) polypeptides of cytochrome b-559 [12]. Other photosystem II polypeptides encoded by chloroplast genes are the 9 kDa phosphoprotein of photosystem II (psbH) [16] and a 24 kDa polypeptide (psbG) [34]. Many of the chloroplast genes for photosystem II polypeptides are clustered (psbB-psbH, psbD-psbC and psbE-psbF; see [12]) and are co-transcribed into large polycistronic mRNAs which then may be subsequently processed into a complex range of transcripts of differing sizes [23, 37].

In addition to these well-characterised polypeptides, photosystem II preparations also contain 4 small polypeptides whose gene location is unknown [20]. These polypeptides have reported molecular masses of 7, 6.5, 5.5 and 5 kDa. The 7, 6.5 and
5.5 kDa polypeptides partition into Triton X-114 and are presumably hydrophobic proteins associated with the thylakoid membrane. The 5 kDa polypeptide is hydrophilic and its amino acid composition has been determined [20]. Nucleotide sequencing has revealed the presence of small open reading frames in chloroplast DNA that appear to be co-transcribed with known photosystem II genes. These include an open reading frame of 38 amino acids co-transcribed with the psbB and psbH genes and two open reading frames of 38 and 40 codons co-transcribed with the psbE and psbF genes for cytochrome b-559 [13]. The fact that these open reading frames are co-transcribed with known photosystem II polypeptides has led to speculation that they may also encode components of photosystem II [5]. To date, however, no evidence has been published to indicate that polypeptides encoded by these open reading frames are components of photosystem II.

In this paper we report the N-terminal amino acid sequence of a previously unidentified photosystem II polypeptide that corresponds to an open reading frame of 38 codons in chloroplast DNA. The transcription of this gene has been analysed and we report that it is co-transcribed with two photosystem II genes, psbE and psbF, as part of a large polycistrionic message.

Materials and methods

Plant growth

Wheat (*Triticum aestivum* cv. Sentry) seeds were sown in Fisons Levington compost and grown in darkness for 7 days at 23 °C. After 7 days plants were either kept in darkness or irradiated with 70 μmol photons m⁻² s⁻¹ from fluorescent lights for 4 and 24 h. Only the top 6 cm of the first leaves were harvested. Leaf material used for thylakoid protein isolation was harvested from 10-day-old plants grown in a greenhouse.

Protein isolation

Thylakoid membranes were isolated from 10-day-old wheat leaves using the homogenisation buffer of Berthold et al. [1]. Photosystem II particles were prepared from washed thylakoid membranes solubilised with Triton X-100 at a detergent to chlorophyll ratio of 25:1 (w/w) as described by Berthold et al. [1]. Photosystem II polypeptides were fractionated by SDS-PAGE using the method of Fling and Gregerson [11], except the resolving gel contained 18% acrylamide and 5 M urea. After electrophoresis gels were soaked in transfer buffer (20 mM Tris, 150 mM glycine, 20% methanol and 0.05% SDS) before electroblotting to polyvinylidene difluoride (PVDF) membrane (Millipore) at 250 mA for 2 h in transfer buffer. After transfer the PVDF membrane was rinsed in double-distilled water for 10 min and stained with 0.1% Coomassie Brilliant Blue-R in 50% methanol for 5 min at room temperature [21]. The membrane was then destained in 50% methanol, 10% acetic acid for 10 min and rinsed in deionised water for 10 min, air-dried and stored at −20 °C.

When required for electroelution polypeptides were visualised by staining with 0.5% Coomassie Brilliant Blue-R in double-distilled water and destaining in double-distilled water. Stained bands were excised from the gels using a razor blade and the polypeptides electroeluted at 50 mA for 2 h [9]. After dialysis of the electroeluted polypeptides against 0.01% SDS for 24 h the samples were freeze-dried and stored at −20°C.

Protein sequencing

The electroeluted 3.2 kDa protein was dissolved in water and precipitated twice with 90% (v/v) ethanol to remove excess Coomassie Brilliant Blue stain, SDS and other UV-absorbing materials. The sample was dried under vacuum, redissolved in trifluoroacetic acid and applied to a glass fibre disc containing 3 mg of Biobrene Plus and precycled according to manufacturer’s instructions. Sequence analysis was performed on an Applied Biosystems 477A Pulsed-liquid Phase Protein Sequencer with an on-line...