Isolation and characterization of cDNA clones encoding a 18.8 kDa polypeptide, the product of the gene psaL, associated with photosystem I reaction center from spinach

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Abstract

Several cDNA clones encoding subunit XI of photosystem I reaction center (PSI-L) have been isolated from two λgt11 expression libraries based on polyadenylated RNA of spinach seedlings illuminated for 4 and 16 h, respectively. The precursor polypeptide made from these recombinant DNAs in vitro can be efficiently imported into isolated spinach chloroplasts. It is correctly processed to the size of the authentic polypeptide and integrates into the photosystem I assembly. The 834 nucleotide sequence of the longest cDNA insert encodes a precursor polypeptide of 24 kDa (216 residues) and a mature protein of probably 18.8 kDa (169 residues). Hydropathy analysis suggests that the polypeptide contains two transmembrane segments. The protein appears to originate in a single-copy gene in spinach and to be decoded from RNA species of ca. 900 bases.

Photosystem I (PSI) is a membrane-spanning, supramolecular complex located in stroma lamellae and at the fringe of grana thylakoids that catalyzes the transfer of electrons from plastocyanin to ferredoxin [4-6, 18, 19]. The reaction center consists of at least 13 polypeptide species, of which five (psaA, psaB, psaC, psaI, psaJ) are encoded by plastid and eight (psaD–psaH, psaK, psaL; one of these genes has not yet been isolated and named) by nuclear genes [8, 9, 10, 13, 15, 32, 33]. The subunits Ia and Ib, designated CPI [34], constitute the innermost, membrane-spanning reaction center core to which peripheral, relatively hydrophilic, components are attached, the subunits II, IV, V, VI and VII at the stromal side and subunit III at the luminal side of the core [12, 16, 22, 32, 33]. The other subunits are more hydrophobic and probably more tightly associated with CPI [5, 8, 13, 26, 30]. Cross-linking studies suggest that subunit III serves as the docking protein.

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X64445.
for the PSI electron donor plastocyanin \([11, 37]\) while subunits II and IV appear to be involved in the interaction with ferredoxin at the stromal (reducing) site of the complex \([1, 38]\).

Much of our knowledge about the function and assembly of the different subunits associated with the PSI core complex has been derived from the analysis and modification of their genes that have been isolated from various plant species and cyanobacteria \([e.g. 21]\). For spinach, genes for all plastid-coded (\(\text{psa}A, \text{psa}B, \text{psa}C, \text{psa}I, \text{psa}J\)) and five subunits of nuclear origin have been isolated (\(\text{psa}D-\text{psa}H\) \([10]\). However, at least one additional polypeptide associated with the PSI core complex with an identical or slightly faster electrophoretic mobility than subunit III, designated PSI-L \([15]\), a minor component of 9 kDa (PSI-M) which generally comigrates with subunits V and VII (PSI-G and PSI-C, respectively), and the gene product of \(\text{psa}K\) were repeatedly observed in PSI preparations from spinach thylakoid membranes \([e.g., 15]\). The genes for these polypeptides are located in the nucleus \([15, 27]\), but have not yet been isolated at all or from spinach. In continuation of studies aimed at understanding the biogenesis of the PSI reaction center in this organism and to identify regulatory processes that control and coordinate their expression, we have isolated and characterized full-length cDNA clones of the gene \(\text{psa}L\).

Fractionation of partially solubilized thylakoid membranes on sucrose gradients demonstrated that a new polypeptide with a slightly faster electrophoretic mobility than subunit III was tightly associated with the PSI core (Fig. 1). Comparison of the polypeptide patterns of our PSI preparations with those of Ikeuchi and Inoue \([15]\) suggested that it could be identical to the 14 kDa polypeptide characterized as a constituent of PSI from spinach by these authors. This polypeptide, designated PSI-L or subunit XI, has been reported to be N-terminally blocked, but an internal, 21-residue amino acid sequence from a partial proteolytic product of this component had been determined \([15]\).

An oligonucleotide derived from this amino acid sequence (5'-GGTGAA/GCCIT-CIATI-GCICCIGC-3') was used to screen our \(\lambda\)gt11 libraries from spinach \([3, 24, 35]\). More than 30 'positive' phage were identified from approximately \(2.5 \times 10^5\) pfu and the DNA of eight of them was isolated. Their inserts were isolated from agarose gels, the five longest with sizes varying from 500 to 900 nucleotide pairs were inserted into pBSC\(^+\) (Strategene, San Diego) and sequenced. The nucleotide sequence of the longest (834 bp) cDNA fragment, referred to as p6SocPSI11-1, is presented in Fig. 2. The deduced amino acid sequence contains a region (nucleotides 555 to 608 corresponding to the amino acid residues 157 to 178; cf. Fig. 2) which is identical to that reported by Ikeuchi and Inoue \([15]\). We conclude therefore that the isolated cDNAs encode subunit XI and PSI, the product of the gene \(\text{psa}L\). Sequence search disclosed an average