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*Plant Gene Register*

# Cloning and Sequencing of a Full-Length cDNA Clone Encoding the PSI-D Subunit of Photosystem I from Barley[1]

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PSI catalyzes the electron transport from reduced plastocyanin to oxidized Fd in higher plants and cyanobacteria. Thirteen polypeptides have been identified in the PSI complex from higher plants (Andersen and Scheller, 1993). Five of the polypeptides are chloroplast encoded (PSI-A, -B, -C, -I, -J) and eight are nuclear encoded (PSI-D, -E , -F, -G, -H, -K, -L, -N). The *psaA* and *psaB* genes encode the two major polypeptides in the PSI complex that bind pigments, the reaction center P700 and the electron acceptors $A_0$, $A_1$, and X. The PSI-C subunit binds the terminal electron acceptors A and B (Høj et al., 1987). Cross-linking experiments have shown that the polypeptide PSI-D encoded by the *PsaD* gene interacts with Fd (Zanetti and Merati, 1987). A cDNA clone encoding PSI-D from spinach has previously been isolated (Lagoutte, 1988). The primary structure of the transit peptide and the hydrophilic character of PSI-D predict an extrinsic polypeptide located on the stromal side of the thylakoid membrane. Extraction of PSI-D from the PSI core with *n*-butanol (Oh-oka et al., 1988) supports this assignment. Reconstitution experiments with PSI-D and PSI-C overexpressed in *Escherichia coli* have shown that PSI-C binding to the PSI-A/PSI-B heterodimer in vitro requires the presence of PSI-D (Li et al., 1991).

A cDNA library of poly(A)$^+$ RNA from light-induced barley (*Hordeum vulgare* L.) seedlings was constructed in the λ ZAP II vector (Stratagene, La Jolla, CA). The library was screened with a 5′-end-labeled oligonucleotide specifying the barley PSI-D (Okkels et al, 1988). A 637-bp long partial cDNA clone was identified. This partial clone was used as a probe in subsequent screens. The insert sizes of 30 positive clones were determined by Southern blotting. Inserts from five possible full-length clones were in vivo excised from the λ phage using the helper phage M13K07 (Short et al., 1988). Sequencing showed that one clone of 835 bp was a full-length cDNA clone (Fig. 1, Table I). The cDNA contains an open reading frame of 618 bp. The amino acid sequence deduced from the cDNA clone contains regions that match the partial amino acid sequences obtained (Scheller et al., 1988). The deduced amino acid sequence also shows high similarity to PSI-D from other plants and cyanobacteria. The

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                    -39                                          -1
                      ATCTCCTCGCCAAGAACCACAAGCTAGAGCGCCACCGCC
1                                                                48
ATG GCC ATG GCC ACG CAA GCC TCG GCG GCG ACG CGC CAC CTG ATC ACC
Met Ala Met Ala Thr Gln Ala Ser Ala ALa Thr Arg His Leu Ile Thr
                                                                 96
GCG GCC TGG TCG CCA TCC GCC AAG CCC CGC CCC GCC ACC CTC GCC ATG
Ala Ala Trp Ser Pro Ser Ala Lys Pro Arg Pro Ala Thr Leu Ala Met
                                              ▼                 144
CCA TCC TCC GCC CGC GGC CCG GCC CCG CTC TTC GCC GCG GCC CCG GAC
Pro Ser Ser Ala Arg Gly Pro Ala Pro Leu Phe Ala Ala Ala Pro Asp
                                                                192
ACC CCC GCA CCC GCC GCG CCG CCG GCG GAG CCC GCC CCC GCG GGC TTC
Thr Pro Ala Pro Ala Ala Pro Pro Ala Glu Pro Ala Pro Ala Gly Phe
                                                                240
GTG CCG CCG CAG CTG GAC CCG TCC ACG CCG TCC CCG ATC TTC GGC GGC
Val Pro Pro Gln Leu Asp Pro Ser Thr Pro Ser Pro Ile Phe Gly Gly
                                                                288
AGC ACC GGC GGG CTG CTC CGC AAG GCC CAG GTC GAG GAG TTT TAC GTC
Ser Thr Gly Gly Leu Leu Arg Lys Ala Gln Val Glu Glu Phe Tyr Val
                                                                336
ATC ACC TGG ACC TCC CCC AAG GAG CAG GTC TTC GAG ATG CCC ACC GGC
Ile Thr Trp Thr Ser Pro Lys Glu Gln Val Phe Glu Met Pro Thr Gly
                                                                384
GGC GCC GCC ATC ATG CGC GAG GGC CCC AAC CTC CTC AAG CTC GCC CGC
Gly Ala Ala Ile Met Arg Glu Gly Pro Asn Leu Leu Lys Leu Ala Arg
                                                                432
AAG GAG CAG TGC CTC GCC CTC GGC AAC CGC CTC CGC TCC AAG TAC AAG
Lys Glu Gln Cys Leu Ala Leu Gly Asn Arg Leu Arg Ser Lys Tyr Lys
                                                                480
ATC GCC TAC CAG TTC TAC CGC GTC TTC CCC AAC GGC GAG GTG CAG TAC
Ile Ala Tyr Gln Phe Tyr Arg Val Phe Pro Asn Gly Glu Val Gln Tyr
                                                                528
CTC CAC CCC AAG GAC GGC GTC TAC CCG GAG AAG GTC AAC GCC GGC AGG
Leu His Pro Lys Asp Gly Val Tyr Pro Glu Lys Val Asn Ala Gly Arg
                                                                576
CAG GGC GTG GGA CAG AAC TTC CGC AGC ATC GGC AAG AAC GTC AGC CCC
Gln Gly Val Gly Gln Asn Phe Arg Ser Ile Gly Lys Asn Val Ser Pro
                                                                625
ATC GAG GTC AAG TTC ACC GGC AAG AAC TCC TTC GAC ATC TAA TCTCGCT
Ile Glu Val Lys Phe Thr Gly Lys Asn Ser Phe Asp Ile STOP

                                                                688
CGTACGTCGTATATGTGCATGCTTATGCGTACGTGCTAGTTCATCGACCGGTAGTTATTGGCG

                                                                751
GGTGGCGATGATGATGGACGTCCTGTAATTTTAAAATTGTCGAGTTTGAATGATGGATTCGGT

                    771
GATAATGGTTGCATCAGGTT(A)25
```

**Figure 1.** Nucleotide sequence of the barley *PsaD* cDNA clone and the deduced amino acid sequence. The maturation site (▼) is indicated.

threonine and valine residues at bp 288 and 316, respectively, are conserved between PSI-D from barley and cyanobacteria, but not between PSI-D from barley and spinach, tomato, or cucumber. This suggests an evolutionary change at these two positions in dicots, but not in monocots.

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The EMBL accession number for the sequence reported in this article is M98254.

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**Table I.** *Characteristics of the PsaD cDNA clone from barley*

Organism:
*Hordeum vulgare* L. cv Svalöfs Bonus.
Location in Genome:
Nuclear genome.
Gene Function:
*PsaD* encodes PSI-D, an extrinsic subunit of PSI, which interacts with Fd and stabilizes the binding of PSI-C to the PSI core.
Techniques:
cDNA screening; double-stranded plasmid sequencing of both strands by the dideoxy chain termination method.
Method of Identification:
Sequence identity to partial amino acid sequence of the PSI-D polypeptide from barley and sequence comparison to PSI-D from spinach, tomato and cucumber.
Expression and Regulation:
Induced by light at the transcriptional level.
(C + G) Content:
Coding region 71.7%.
Structural Features of the Protein:
Open reading frame of 205 amino acid residues encoding a hydrophilic precursor protein with a calculated $M_r$ of 21,933. The mature protein (162 amino acids) has a calculated $M_r$ of 17,559 and an isoelectric point of 10.17.
Antibodies:
Polyclonal antibodies against PSI-D from barley are available.
Subcellular Location:
Chloroplast thylakoid membrane.

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