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Molecular Cloning and Characterization of a cDNA for the PSI-H Subunit Homolog of Photosystem I in Chinese Cabbage

Joon-Yung Cha, Young Jin Choi, Hyoshin Lee*, Ki-Yong Kim**, Geun Je Park**,
Jinki Jo*** and Daeyoung Son

배추로부터 광계 I의 PSI-H Subunit Homolog의 클로닝 및 분자생물학적 특성 연구

차준영 · 최영진 · 이효신* · 김기용** · 박근제** · 조진기*** · 손대영

적 요

식물의 광합성에 관여하는 광계 I 의 protein subunit들의 연구는 최근까지도 극히 미약한 실정이며, 각각의 subunit들의 특성 또한 일부만이 밝혀져 있다. 본 연구진은 배추의 cDNA library로부터 식물에만 존재하는 subunit 중의 하나인, PSI-H subunit을 암호화하는 유전자인 *bpsaH*를 분리하였다. 이 유전자는 총 633 bp의 염기로 구성되어 있으며, 염기서열로부터 추정되는 분자량은 약 15,400이었고 등전점은 9.91이었다. 배추 PSI-H subunit의 아미노산 서열을 다른 식물체 유래의 단백질들과 비교분석한 결과, 시금치의 PSI-H와 가장 높은 유사성 (79.3%)을 나타내었다. 또한 *bpsaH*의 조직 특이적 발현 양상을 조사한 결과, 광합성 조직인 잎에서는 강하게 발현된 반면 꽃봉우리에서는 약하게 발현되었으며, 비광합성 조직인 뿌리에서는 전혀 발현되지 않았다.

(**Key words** : Photosynthesis, Photosystem I, psaH, *Brassica campestris*)

I. INTRODUCTION

One of distinct mechanisms of green plants is photosynthesis, which converts light energy into chemical energy in the form of reducing power (as NADPH or NADH) and ATP. These energy sources make carbohydrates and other organic compounds from CO_2 and water, and related directly with herbage biomass. Higher plants contain two different reaction center complexes, photosystem I (PSI) and photosystem II (PSII), located in the thylakoid membrane of

경상대학교 식물분자생물학 및 유전자조작연구소(Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju 660-701, Korea)
*임업연구원 생물공학과(Division of Biotechnology, Korea Forest Research Institute, Omokchun-dong, Suwon, 441-350, Korea)
**축산기술연구소 (National Livestock Research Institute, Suwon 441-350, Korea)
***경북대학교 동물공학과 (Department of Animal Science and Biotechnology, Kyungpook National University, Daegu 702-701, Korea)
Corresponding author : Prof. D. Son, Address : Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju 660-701, Korea Tel : (055)751-6028 E-mail : dyson@nongae.gsnu.ac.kr

chloroplast. PSI carries out the photoreduction of ferridoxin by reduced plastocyanin, and contains a large number of protein subunits as PSII. Fifteen protein subunits, including PSI-A through to PSI-N and PSI-U were identified in the PSI complex (Hallick, 1989; Sheller and Møller, 1990; Okkels et al., 1991; Chitnis, 2001). The genes encoding these subunits are designated *psaA* through *psaN* and *psaU*, respectively. The subunits PSI-A and PSI-B, encoded by the chloroplast genes *psaA* and *psaB*, have been shown to bind P700 pigment as well as the primary and secondary electron acceptors A_0, A_1 and F_X. PSI-C, the product of chloroplast gene *psaC*, has a central role in binding the tertiary electron acceptors F_A and F_B. The nuclear encoded subunits PSI-D and PSI-E are involved in the binding of ferredoxin, and PSI-F provide electrostatic interactions for the binding of plastocyanin. PSI-H subunit encoded by a nuclear gene *psaH* has about 10 kDa molecular mass and may contribute to the interactions between PSI and ferredoxin. PSI-I, PSI-J and PSI-M subunit encoded by a chloroplast gene *psaI*, *psaJ* and *psaM*, respectively, are small hydrophobic proteins of PSI and contain one transmembrane helix each (Almog et al., 1992; Bryant, 1992; Malkin and Niyogi, 2000). Only three subunits, PSI-G, PSI-H, and PSI-N, are found in plants and other remaining subunits are shared between cyanobacteria and plants. However, the role of the three plant specific subunits is less understood than others.

Several cDNA or genomic clones encoding PSI-H have been isolated and analyzed from spinach (Steppuhn et al., 1989), barley (Okkels et al., 1989), two species of Nicotiana (Hayasida et al., 1990), rice (de pater et al., 1990), Chlamydomonas (Franzén et al., 1989) and Arabidopsis (Naver et al., 1999). Recent data has shown the PSI-H can be cross-linked with PSI-D, PSI-I and PSI-L (Jansson et al., 1996) and is required for stable accumulation of PSI and efficient electron transfer in the complex (Naver et al., 1999).

In this study, we isolated a cDNA clone, *bpsaH*, which is homologus to *psaH* from Chinese cabbage. The structure and the expression level in different organs of *bpsaH* were determined in order to examine the role of PSI-H subunit in the PSI complex.

II. MATERIALS AND METHODS

1. Plant material

Brassica compestris L. ssp. *pekinensis* (from Seoul Seed Co., Korea) was grown in a greenhouse.

2. Construction of cDNA library

Total RNA was prepared from 3 g of flower buds according to the protocol described previously (Ausubel et al., 1992). Poly(A^+) RNA was isolated from the total RNA using an mRNA purification kit (Pharmacia, USA) according to the manufacture's protocol. The cDNA library was constructed into the pBluescripts KS(+) vector as described (Lim et al., 1994). A plasmid library with approximately 5×10^7 recombinants was obtained.

3. DNA sequencing and computer analysis of protein sequences

Nucleotide sequencing was performed by the dideoxy chain termination method (Sanger et al.,

1977) on double-stranded plasmid DNA using Sequenase (USB). Sequence comparisons were performed in PIR and SWISS-PROT databases using the search program FASTA (Pearson and Lipman, 1988).

4. Northern blot analysis

Total RNA was denatured by formaldehyde and subjected to electrophoresis on 1.2% agarose gels containing formaldehyde as previously described (Sambrook et al., 1989). The RNA was blotted on to nylon membrane (Hybond-N+, Amersham) and prehybridized at 42℃ for 2 h in 50% formamide, 50 mM sodium phosphate buffer (pH 6.5), 5 × Denhardt's solution (1× Denhardt's solution is 0.02% w/v Ficoll, 0.02% w/v PVP, 0.02% w/v BSA), 5× SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate), 0.1% SDS, and 100 μg/mℓ denatured salmon sperm DNA. Hybridization was conducted at 42℃ overnight with a probe which was prepared by the megaprime DNA labelling system (Amersham) using [α-^{32}P]dATP. The RNA blot was washed with 2× SSC, 0.1% SDS at 42℃ twice for 30 min and then with 0.1× SSC, 0.1% SDS at 65℃ for 30 min. The blot was visualized by autoradiography at −70℃ using X-ray film and an intensifying screen.

5. Southern blot analysis

Genomic DNA was prepared from young leaves (Ausubel et al., 1992). DNA samples (10 μg) were digested with appropriate restriction endonucleases and separated by electrophoresis on 0.8% agarose gel. The gel was blotted to a nylon membrane, prehybridized and hybridized with ^{32}P-labeled cDNA probe. After hybridization, the filter was washed and subjected to autoradiography as described above.

Ⅲ. RESULTS AND DISCUSSION

One of the randomly selected clones, *bpsaH*, from a flower bud cDNA library of *B. compestris* exhibited high homology to the PSI-H subunit of spinach at the amino acid level. The composite nucleotide sequence and the deduced amino acid are shown in Fig. 1. The entire sequence was 633 nucleotides, including 18 adenines of the poly(A) tail. The sequence contained an open reading frame of 435 nucleotides which gave a deduced amino acid sequence of 145 residues encoding a precursor protein with a calculated molecular mass of 15,408 Da. The first available initiation codon was at position 49, which gave the sequence of AAAC*AUG*GC, compatible to the consensus sequence for the start of translation in plants (Lütcke et al., 1987). Presuming that the mature polypeptide is not C-terminally processed, the molecular mass of the mature polypeptide and the N-terminal transit peptide was calculated to be 10,371 and 5,037 Da. The amino acid sequence deduced from the cDNA sequence is highly similar to those of previously reported PSI-H proteins (Fig. 2), strongly suggesting that the isolated cDNA most likely encodes a PSI-H subunit of Chinese cabbage. The sequence similarity among higher plants are highly conserved (> 70%); however the *C. reinhardtii* protein is only about 33% identical in sequence to those of higher plants. Transit peptides of various precursors of chloroplastic proteins are known to share several features; they are very rich in positively charged amino acids and small hydrophobic amino acids such as alanine and

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                                                        CAA
CAG CAA CAA GAG ATA AGT CTT TGT TGT GTG TTT GGT CTG AGA AAC   -1

ATG GCA TCT TTT GCA ACC ATC GCC GCC GTT CAA CCA TAC TCC GCC    45
 M   A   S   F   A   T   I   A   A   V   Q   P   Y   S   A    15

GTG AAA GGA CTC GGA GGA AGC TCT CTC ACC GGA GCT AAG CTC TTC    90
 V   K   G   L   G   G   S   S   L   T   G   A   K   L   F    30

ATC AAG CCT TCT CGC CAA AGC TTC AAA CCC AAA TCC ACA AGG GCT   135
 I   K   P   S   R   Q   S   F   K   P   K   S   T   R   A    45

GGT GCC GTG GTG GCC AAG TAT GGA GAC AAA AGT GTT TAC TTT GAT   180
 G   A   V   V   A   K   Y   G   D   K   S   V   Y   F   D    60

TTA GAA GAT TTA GGT AAC ACA ACA GGA CAA TGG GAT TTG TAT GGA   225
 L   E   D   L   G   N   T   T   G   Q   W   D   L   Y   G    75

TCT GAT GCT CCT TCT CCT TAC AAC CCA CTT CAG AGC AAG TTC TTT   270
 S   D   A   P   S   P   Y   N   P   L   Q   S   K   F   F    90

GAG ACA TTT GCT GCT CCT TTC ACA AAG AGA GGT TTG CTT CTC AAG   315
 E   T   F   A   A   P   F   T   K   R   G   L   L   L   K   105

TTT TTG ATT CTT GGA GGA GGC TCT TTG CTT ACT TAT GTC AGT GCT   360
 F   L   I   L   G   G   G   S   L   L   T   Y   V   S   A   120

TCC TCA ACT GGC GAT GTT CTT CCT ATC AAG AGA GGT CCT CAA GAG   405
 S   S   T   G   D   V   L   P   I   K   R   G   P   Q   E   135

AAG CCT AAG CTC GGT CCT CGC GGC AAG CTT TAA ATT AGC TTT CTA   450
 K   P   K   L   G   P   R   G   K   L   *                   145

AAT CCC TTG AAA ACA TCA GAA ACT TTC TTC CCA AGT CTC TGA ACT   490
CCT GTT GTT ACT GAT CTA TGT ATA TTG TCT TGT GTT AAT TGC TAT   540
GAT GAA TCA ATG AAA AAA AAA ATT TGC AAA AAA AAA AAA AAA AAA   585
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Fig. 1. Nucleotide and deduced amino acid sequence of cDNA encoding PSI-H homolog from Chinese cabbage. The deduced amino acid residues are written below the nucleotide sequence. The stop condon is marked with three asterisks, and the region corresponding to the putative polyadenylation signal is underlined.

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BC  MASFATIAAV QPYSAVKGLG GSSLTGAKLF IKPSR--QSFKP KSTRAGAVV▼A  50
SO  ...L..L... ..-TTL...A ...IA.T..H ...A.--....L NNV.S..I. .  49
NS  ...L.AF... ..TTN....A ...I..T..H L.S..--LNL.. TKS...P.. .  50
NT  ...L..LT.. ..TTTI...- -RAIA.T..N V.S..--LNL.. SKS..... .  50
OS  ...LV---.. ..-V.....A ...IS.R..A VR..P--RALCR TTR.RA... .  46
HV  ...LV---.. ..-A.....S ...IS.R..A VR..SAAVSRST RRA.GA... .  48
CR  ..LV.----- ---------- -RPVLS.RVA --A..----PRV AARK.VR.S .  30

BC  KYGDKSVYFD LEDLGNTTGQ WDLYGSDAPS PYNPLQSKFF ETFAAPFTKR  100
SO  .......... ...IA..... ..V..... ...S..... ..........  99
NS  .......... .......... .......... ...S..... ..........  100
NT  .......... .......... .......... .......... ..........  100
OS  ...E..... ...I..... ....A..... .......... ....G.....  96
HV  ...E..... .D.IA..... .......... ...G..... N.........  98
CR  ...EN.R... .Q.ME....S ..M..V.EKK R.PDN.A... TQATDIISR.  80

BC  GLLLKFLILG G-GSLLTYVSA SSTGDVLPIK RGPQEKP---KLG -PRGKL  145
SO  .......... .-.......... NAPQ.....T ....QP.---... -....I  144
NS  .......... .-..T.A.F.S TAS..I.... K...LP.---... -....I  145
NT  .......... .-..T.A.F.S TAS..I.... K...LP.---... -....I  145
OS  .......... .-...VA.... .ASP.L.... K..HVP.---TP. -....-  140
HV  .......LI. .-...VA.... .ASP.L.... K...LP.---TP. -....-  142
CR  ES.RALVA.S .IAAIV..GLK GAKDAD...T K...TTGENG.G. SV.SR.  130
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Fig. 2. Comparison of the deduced amino acid sequences of the psaH gene from various species. Gaps are inserted to obtain the highest homology. The amino acid residues that are the same as bpsaH are denoted as a dot. The arrow indicates the processing site of the precursor. Abbreviations: BC, *Brassica compestris*; SO, *Spinacia oleracea* (Steppuhn et al., 1989); NS, *Nicotiana sylvestris* (Hayashida et al., 1992); NT, *Nicotiana tabacum* (hayashida et al., 1992); OS, *Oriza sativa* (de Pater et al., 1990); HV, *Hordeum vulgare* (Okkels et al., 1989); CR, *Chlamydomonas reinhardtii* (Franzén et al., 1989).

valine, and lack acidic residues (Keegstra et al., 1989). All these features are found in Chinese cabbage PSI-H homolog.

To gain an insight into the biological role of *bpsaH*, we examined the expression of the gene by northern blot analysis using total RNA isolated from various tissues. As shown in Fig. 3, a single band of 0.6kb was expressed highest in leaf tissue, less in flower buds but undetectable in root tissue. Considering that the cloned gene is likely that of a PSI-H subunit, the lack of transcript in non photosynthetic

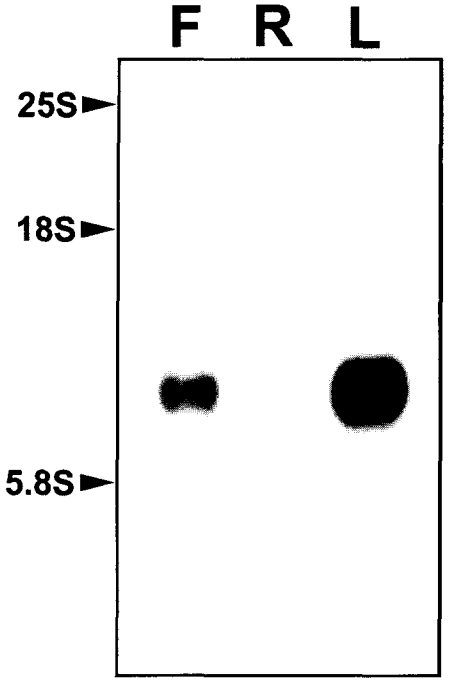

Fig. 3. Expression of the *bpsaH* gene in different organs of Chinese cabbage. Total RNA (20 μg) from flower buds (lane F), roots (lane R) and leaves (lane L) were size-fractionated and hybridized with ^{32}P-labeled *bpsaH* cDNA. The migration of ribosomal RNA are indicated at the left of the figure.

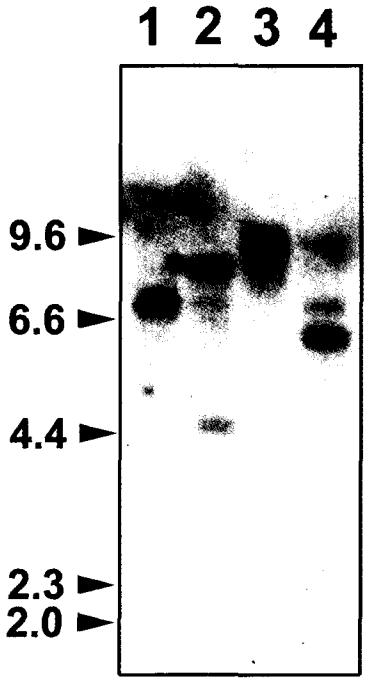

Fig. 4. Southern blot analysis of Chinese cabbage genomic DNA. Genomic DNA digested with *Bam*HI (lane 1), *Eco*RI (lane 2), *Kpn*I (lane 3) and *Pst*I (lane 4) were size-fractionated and hybridized with ^{32}P-labeled *bpsaH* cDNA.

tissue is not surprising.

The approximate copy numbers of *bpsaH* in the Chinese cabbage genome were estimated by southern blot analysis. Genomic DNA (10 μg) were digested separately with *Bam*HI, *Eco*RI, *Kpn*I, and *Pst*I, none of which cleaved the *bpsaH* cDNA insert. The resulting DNA fragments were hybridized with ^{32}P-labeled entire cDNA insert. As shown in Fig. 4, the southern blot analysis revealed that *bpsaH* maybe encoded by two genes in the Chinese cabbage genome.

Recently, it was reported that PSI-H is essential for efficient electron flow in the PSI complex. Furthermore, PSI-H is required for interaction with PSI-L, stabilization of F_X, and the overall stability of the PSI complex (Naver et al., 1999). Lunde et al. (2000) also revealed that PSI electron transport *in vitro* was reduced by approximately 40% and plastoquinone pool was more reduced in the absence of PSI-H or PSI-L. Nevertheless, the precise mechanism by which PSI-H affects electron transport is still unraveled. To dissect this mechanism of PSI-H the cloned *bpsaH* cDNA will serve as an important tool.

Ⅳ. ABSTRACT

PSI-H is an intrinsic membrane protein known to be essential for efficient electron flow in PSI complex. We isolated a cDNA clone encoding a PSI-H subunit homolog from Chinese cabbage (*Brassica campestris* L.). The cDNA, designated bpsaH, had an insert of 435 bp and a full open reading frame that would encode a protein of 145 amino acids. The amino acid sequence deduced from the cDNA sequence is 79.3% identical to that of spinach, suggesting the cDNA most likely encodes Chinese cabbage PSI-H subunit. The *bpasH* was expressed at high level in leaf tissue and low level in flower bud, whereas it was undetectable in root tissue.

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