Volume 287, number 1,2, 5–9 FEBS 09954 August 1991

*ADONIS* 0014579391006648

# Identities of four low-molecular-mass subunits of the photosystem I complex from *Anabaena variabilis* ATCC 29413

## Evidence for the presence of the *psa*I gene product in a cyanobacterial complex

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Received 26 March 1991; revised version received 14 May 1991

Photosystem I (PSI) complex of *Anabaena variabilis* ATCC 29413 consists of at least 11 subunits, 9 of which are resolved by high resolution gel electrophoresis. N-terminal amino acid sequences of the four subunits with molecular masses of 6.8, 5.2, 4.8 and 3.5 kDa were determined. Based on the sequence homology, the 3.5 kDa subunit was revealed to correspond to PSI-I (the gene product of *psa*I), which had so far been detected only in higher plant PSI complexes. The 6.8 kDa protein and 4.8 kDa protein were identified as gene products of *psa*K and *psa*J, respectively. The 5.2 kDa protein was homologous to a 4.8 kDa subunit of PSI of the thermophilic cyanobacterium *Synechococcus vulcanus*, suggesting that this protein is a component of PSI in cyanobacteria.

Cyanobacterium; Photosystem I; Protein sequence; *psa*I; *Anabaena variabilis* ATCC 29413

## 1. INTRODUCTION

PSI consists of a number of protein subunits. In higher plants, 17 subunits have been detected in the PSI-LHCI complex [1–7]. Five subunits (PSI-A, PSI-B, PSI-C, PSI-I and PSI-J) are encoded by the chloroplast genome, while the others (PSI-D, PSI-E, PSI-F, PSI-G, PSI-H, PSI-K, PSI-L, 9 kDa protein and four LHCI apoproteins (Type I–IV)) are encoded by the nuclear genome. Current information about their function is limited to a few subunits [8]. A heterodimer of PSI-A and PSI-B is believed to house the photochemical reaction center consisting of P700, acceptor $A_0$, $A_1$ (phylloquinone), and iron–sulfur center X [9,10]. PSI-C ligates iron–sulfur centers A and B [11–13]. PSI-D binds ferredoxin and PSI-C [14,15]. PSI-F binds plastocyanin [16]. Function of the other subunits has not yet been established.

Comparative study of the subunit composition of the PSI complex from cyanobacteria and higher plant provides insight into PSI evolution as well as subunit function. By protein sequencing, we have been performing the comprehensive studies to list up all the PSI subunits from spinach [4,5,7] and the thermophilic cyanobacterium *Synechococcus vulcanus* [17,18]. Through us and others [19–22], it has been shown that many PSI subunits of cyanobacteria correspond to those of higher plants. However, in the range of the low-molecular-mass proteins (<9 kDa), the PSI-I protein has not been detected in any cyanobacterium so far. In addition, the 4.8 kDa PSI subunit of the thermophilic cyanobacterium *Synechococcus vulcanus* has not been identified in any other PSI complex.

In order to gain insight into the nature of the cyanobacterial PSI complex, we have purified the PSI complex from another cyanobacterium, *Anabaena variabilis* ATCC 29413. This filamentous cyanobacterium has the capacity to fix nitrogen in specialized cells called heterocysts. This organism also has the capacity to grow completely heterotrophically with fructose as a carbon source [23], indicating that PSI is not required for its growth. With the development of gene transfer systems for this organism [24], it has become possible to create gene interruptions in the PSI genes of *Anabaena* (Mannan, R.M., Nyhus, K. and Pakrasi, H.B., unpublished observations).

In the current report we focus on the identification of the low-molecular-mass proteins from the PSI complex of *Anabaena*. After efficient lipid extraction of the highly purified PSI complex, we could demonstrate by means of direct protein sequencing the presence of PSI-I as well as PSI-J, PSI-K and a 5.2 kDa subunit in the low-molecular-mass region.

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*Abbreviations:* CPI, chlorophyll–protein complex I of photosystem I reaction center; LHCI, light-harvesting chlorophyll complex associated with photosystem I; LDS, lithium dodecylsulfate; PSI, photosystem I; PSI-X, gene product of *psa*X; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis

## 2. MATERIALS AND METHODS

### 2.1. *PSI preparation*

*Anabaena variabilis* ATCC 29413 was grown in AA/8 media [25] bubbled with air at 30°C. Isolation of PSI was carried out according basically to [26] with slight modifications [27]. Thylakoids were solubilized with dodecylmaltoside and fractionated by two cycles of ion exchange column chromatography using Pharmacia Mono Q column. The PSI fraction eluted from the second column was concentrated by precipitation with 20% polyethyleneglycol and ultracentrifugation at 200000 × *g* for 4 h, and used for protein analysis.

### 2.2. *SDS-PAGE and sequencing*

PSI proteins were analyzed by SDS-urea-PAGE with high resolution in low-molecular-mass region according to Ikeuchi and Inoue [28]. For analytical purposes, the PSI preparation was dissolved in 2% (w/v) LDS, 60 mM dithiothreitol, 60 mM Tris-HCl (pH 8.5) at a chlorophyll concentration of 0.8 mg/ml and heated at 70°C for 1 min or 100°C for 3 min. Alternatively the PSI complex was treated with methanol/ether (1:9 v/v) to extract lipids [29], and then dissolved in 2% (w/v) LDS, 60 mM dithiothreitol, 60 mM Tris-HCl (pH 8.5) at the same chlorophyll concentration. For protein sequencing, the methanol/ether-treated sample was put in a wide sample lane of the same gel, electrophoresed, electrotransferred to ProBlot membrane (Applied Biosystems), stained with Amido black 10B (Bio-Rad), and then subjected to protein sequencing basically according to Ikeuchi et al. [30]. The lipid extraction improved the resolution of low-molecular-mass bands and abolished wavy migration of wide bands, which often happens if samples contain some lipids. This improvement was essential in preparative gel electrophoresis for sequencing the low-molecular-mass proteins, although it is not so clearly demonstrated in an analytical gel (e.g. Fig. 1), where a lesser amount of the sample was enough for detection of protein bands. The combination of the ProBlot (an improved polyvinylidene difluoride membrane) and the sample pretreatment with methanol/ether greatly improved the sequence determination of the small proteins.

## 3. RESULTS

The resolution of the components of the PSI complex from *Anabaena* by SDS-urea-PAGE is shown in Fig. 1. Without a heat treatment, many of the lower molecular weight proteins enter the gel. After treatment of the complex at 70°C, more of the higher molecular weight proteins move into the resolving portion of the gel, with concurrent release of pigments. Upon heating at 100°C, proteins of 15, 12, 6.8 and 5.2 kDa were greatly diminished and CPI apoproteins formed an aggregate at the top of the resolving gel. This might indicate that these proteins are co-aggregated with CPI apoproteins. The methanol/ether treatment removed pigments and lipids without extracting proteins, although aggregated CPI apoproteins did not enter the stacking gel.

This PSI complex consists of 9 visible protein bands of apparent molecular masses of 60, 16, 15, 14, 9, 6.8, 5.2, 4.8 and 3.5 kDa (Fig. 1). The thick, fuzzy band at 60 kDa is actually two proteins, PSI-A and PSI-B. PSI-C was often found as a diffuse band around 11 kDa, which is not discernible in Fig. 1. Thus there is a total of eleven proteins in this complex. All other stained bands were identified as shown in Fig. 1 by direct sequencing.

Relative abundance of the visible components based on relative staining vs apparent molecular mass was

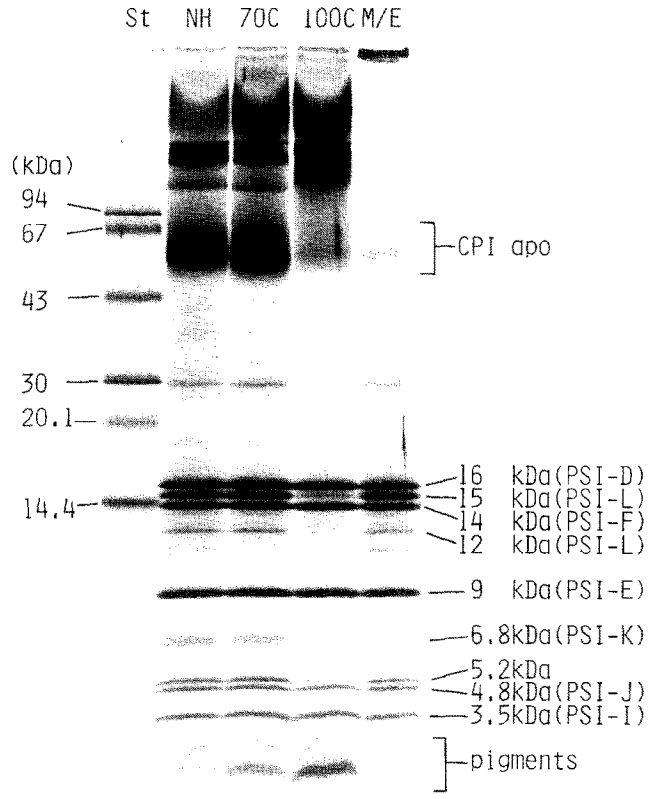
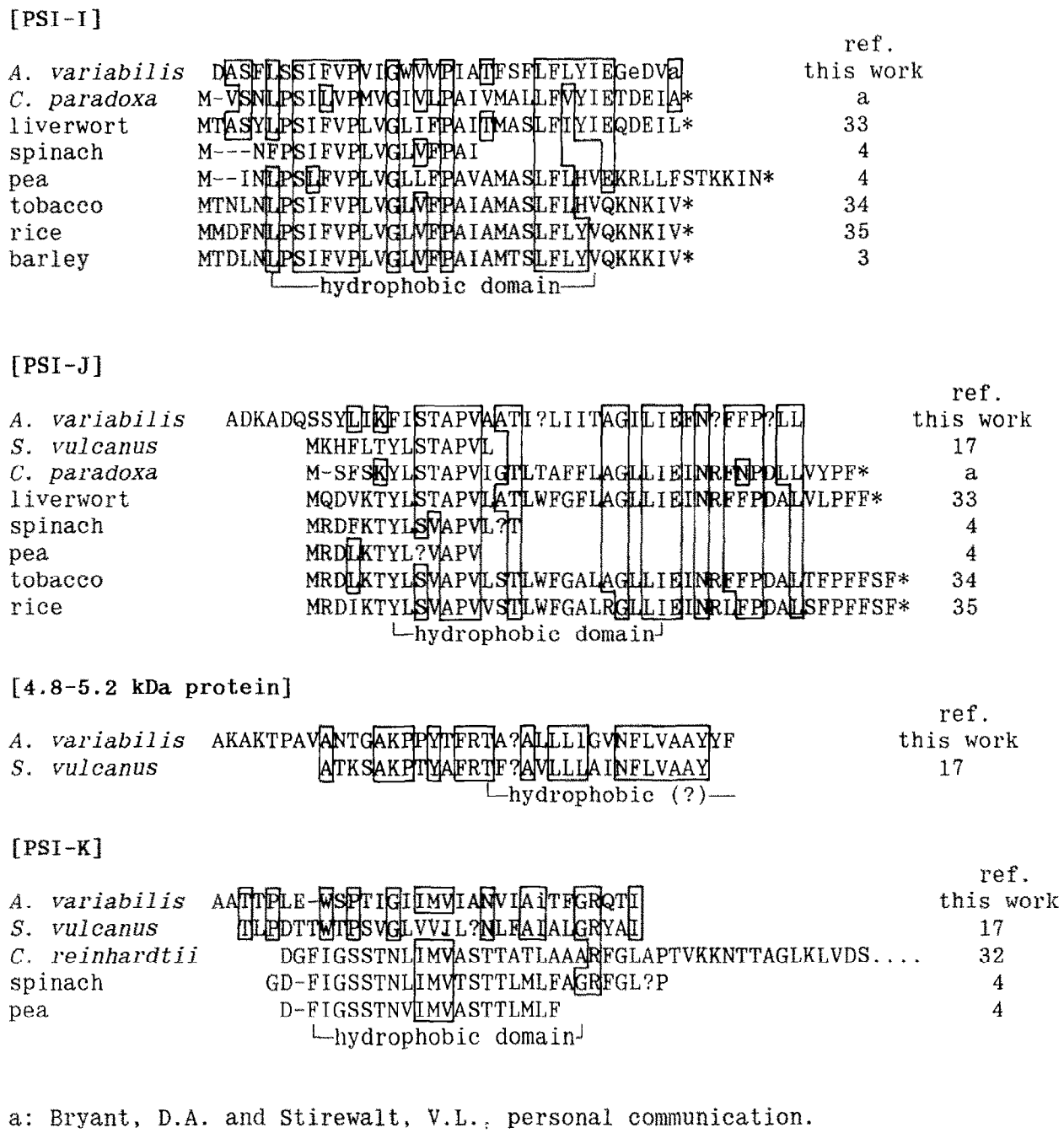



Fig. 1. Polypeptide composition of PSI complex from *Anabaena* sp. ATCC 29413. Pretreatments of PSI complex: NH, not heated; 70C, heated at 70°C for 1 min; 100C, heated at 100°C for 3 min; M/E, extracted with methanol/ether (1:9). Protein bands were identified by direct sequencing. Bands of 30 kDa and 12 kDa band are breakdown products of PSI-B and PSI-L, respectively. The stacking gel is also shown in the figure because some CPI aggregates stayed on the top of or in the stacking gel. 'St' stands for molecular standards (phosphorylase *b* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa) and α-lactoalbumin (14.4 kDa)).

determined as follows: 16 kDa; 15 kDa; 14 kDa; 9 kDa; 6.8 kDa; 5.2 kDa; 4.8 kDa; 3.5 kDa = 1; 0.5–0.6; 0.8; 1; 0.3; 0.4; 0.4–0.5; 0.8. In contrast to the rough one-to-one ratio of 16 kDa, 14 kDa and 9 kDa components, the relative abundance of 6.8 kDa, 5.2 kDa and 4.8 kDa components was low. Since a similar staining ratio was observed in the *Synechococcus* PSI complex [17], the apparent low abundance of the small proteins might be due to inherently poor binding of stain.

By sequencing the 3.5 kDa protein on the ProBlot membrane, 35 amino acids were determined from N-terminus without pretreatment for deblocking (Fig. 2). Sequence alignment with known PSI proteins revealed that this protein is highly homologous to PSI-I. This is the first evidence that PSI-I is associated with the PSI complex in cyanobacteria, since PSI-I was not detected in the PSI complex from *Synechococcus vulcanus* [17]. Based on the sequence alignment, the *Anabaena* PSI-I seems to have lost an initial Met residue, whereas PSI-I




a: Bryant, D.A. and Stirewalt, V.L., personal communication.

Fig. 2. N-terminal sequence of *Anabaena* 6.8 kDa, 5.2 kDa, 4.8 kDa and 3.5 kDa aligned with known sequences. Amino acid residues are shown as one-letter-code. Conserved residues are boxed. Residues shown with small letters were not conclusively determined. Sequences with '*' (termination codon) were deduced from gene sequences, while the others were obtained by protein sequencing. Exceptionally, only N-terminal region of *Chlamydomonas* PSI-K is presented, although the full sequence is known on the basis of its gene sequence [32]. *A. variabilis*, *Anabaena variabilis* ATCC 29413; *C. paradoxa*, *Cyanophora paradoxa*; *S. vulcanus*, *Synechococcus vulcanus*; *C. reinhardtii*, *Chlamydomonas reinhardtii*.

of higher plants retains the Met residue, which is probably blocked by a formyl group [3,4]. Since sequencing cycles after the Ala residue at position 35 did not show any significant signals, the *Anabaena* PSI-I seems to end at this position as in most other organisms. With this assumption, the molecular mass of the *Anabaena* PSI-I is estimated as 3580 Da, which agrees well with the apparent value as estimated by SDS-PAGE (3.5 kDa). Assuming that the initial Met was removed in *Anabaena*, homology of *psaI* at the amino acid level is 47% between *Anabaena* and liverwort, 45% between *Anabaena* and *Cyanophora*, and 36% between *Anabaena* and tobacco. An internal hydrophobic stretch of more than 25 amino acids, which is much more conserved than hydrophilic ends, may span the thylakoid membrane.

N-terminal sequence of the 4.8 kDa protein was revealed to be homologous to the known PSI-J sequences from *Synechococcus vulcanus*, *Cyanophora paradoxa* and green plants (Fig. 2). Interestingly, only the *Anabaena* PSI-J carries extra residues at the N-terminus region. These residues may explain the difference in apparent molecular mass estimated by SDS-PAGE between the *Anabaena* PSI-J (4.8 kDa) and others such as *Synechococcus* (4.1 kDa), pea (4.1 kDa) or spinach (4.1 kDa) [4,17]. Other characteristics of the *Anabaena* PSI-J are the lack of an initial Met residue and the free amino group of the next Ala residue, whereas both *Synechococcus* and higher plant PSI-J proteins retain the Met residue with a blocked amino group. Since PSI-J has an internal hydrophobic domain, which is fairly conserved between *Anabaena* and higher plants, this protein might also span the thylakoid membrane.

The 5.2 kDa protein gave an N-terminal sequence (Fig. 2) which was significantly homologous to the 4.8 kDa protein of *Synechococcus vulcanus* [17]. A hydrophobic domain with a preceding Arg residue is conserved between *Anabaena* and *Synechococcus*: this domain may span the thylakoid membrane as suggested before [17]. The major difference in the sequence between *Anabaena* and *Synechococcus* is the 8 amino acid N-terminal extension in the *Anabaena* protein. This may account for the difference in the apparent molecular masses (4.8 kDa vs 5.2 kDa), although the C-terminal end sequences of the proteins have not been determined.

Sequencing of the 6.8 kDa protein revealed that this is significantly homologous to the *Synechococcus* 6.5 kDa protein (Fig. 2). Although it was proposed recently that the *Synechococcus* 6.5 kDa protein is homologous to PSI-K from *Chlamydomonas* and higher plants [2,4,31,32], the similarity between the *Anabaena* 6.8 kDa protein and PSI-K of plants is low. The protein has a hydrophobic domain, and amino acids may be conservative replacements instead of identical residues.

## 4. DISCUSSION

Of the four proteins discussed in this paper, *Anabaena* shares three with higher plants. The fourth protein, at 5.2 kDa, has been found only in another cyanobacterium, indicating that it might be specific to this group of organisms.

The N-terminal sequence of the *Anabaena* 3.5 kDa protein is the first evidence to demonstrate that PSI-I protein is a PSI component in cyanobacteria as well as in higher plants. In addition, the *psaI* gene was recently cloned from cyanelle DNA of *Cyanophora paradoxa* (Bryant, D.A. and Stirewalt, V.L., personal communication). Thus, it can be concluded that PSI-I is one of the evolutionarily-conserved subunits in the PSI complex. Since only the internal hydrophobic segment is conserved, this region may be functionally significant. Targeted mutagenesis of *psaI* in *Anabaena* may provide a test of its function.

PSI-I has not yet been detected in other cyanobacteria. Very recently we found a 3.5 kDa protein in the *Synechococcus* PSI complex after the methanol/ether treatment (Ikeuchi, M., Sonoike, K., Koike, H. and Inoue, Y., unpublished). However, its N-terminal sequence was not significantly homologous to PSI-I, suggesting the presence of a new component instead of PSI-I. A homologous protein to this *Synechococcus* protein was also detected in the PSI complex from *Synechocystis* sp. PCC 6803, whereas the PSI-I protein was not (Ikeuchi, M., Pakrasi, H.B. and Inoue, Y., unpublished). These might mean that PSI-I is not tightly bound to PSI complex in these cyanobacteria. More careful preparation of intact PSI complex from various cyanobacteria would be needed for further biochemical study of PSI-I.

*Acknowledgements:* Authors thank Drs D.A. Bryant and V.L. Stirewalt (Pennsylvania State University, USA) for kindly providing their unpublished sequence data from *Cyanophora paradoxa* and Dr J.S. Okkels (Royal Veterinary and Agricultural University, Denmark) for kindly providing preprints. Authors also thank Mr N. Kaneda for his technical assistance. The work in Y.I.'s lab was supported by a Grant on Photosynthetic Sciences awarded to the Institute of Physical and Chemical Research (RIKEN) by the Science and Technology Agency (STA) of Japan, and partly by a Grant on Frontier Research Program at RIKEN awarded by STA. Work in H.P.'s lab was supported by funds from National Institutes of Health, USA (GM41841) and Lucille B. Markey Charitable Trust. K.N. was supported by an Olin Fellowship and a National Science Foundation Fellowship at Washington University, USA.

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