PHOTOSYNTHETICA 41 (1): 1-7, 2003

REVIEW

The PsbH protein of photosystem 2

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Abstract

The PsbH protein belongs to a group of small protein subunits of the photosystem 2 (PS2) complex and genes encoding PsbH homologues have been so far found in all studied oxygenic phototrophs. This single helix membrane protein is important for the proper function of the PS2 acceptor side and for stable assembly of PS2. Its hypothetical function as an analogue of the H subunit of the bacterial reaction centre as well as a putative role of its phosphorylation is evaluated.

Additional keywords: chloroplast; cyanobacterium; D1 protein; phosphorylation; photosynthesis.

Identification of the protein

Photosystem 2 (PS2) of higher plants, algae, and cyanobacteria is a membrane pigment-protein complex catalysing oxidation of water and reduction of plastoquinone. Its core consists of nearly 10 protein subunits of variable size. Two pairs of homologous polypeptides D1-D2 and CP47-CP43 represent the larger ones. The first pair binds redox-active functional groups of the reaction centre (like P680, pheophytin, and quinones Q_A and Q_B) performing charge separation while the second pair binds chlorophyll (Chl) molecules needed for energy transfer to the reaction center. There are also a number of low molecular mass proteins mostly with unknown function even though it is assumed that they are important for the optimisation of electron and energy transfer and for assembly of the complex (for review see Hankamer *et al.* 1997).

Bennett (1977, 1979) has originally found in thylakoids of higher plants the PsbH protein, the product of the psbH gene, as 10-kDa phosphoprotein. Its phosphorylation occurs on the threonine residue similarly to LHC2 proteins (Bennett 1977) and this phosphate group could be removed by trypsin (Bennett 1980). The protein has been initially considered as part of the ATP synthase due to its co-migration with the DCCD-reactive CFo subunit III (Alfonso *et al.* 1980), as a small subunit of the cytochrome b_6f complex (Süss 1981), or as the α-subunit of cytochrome *b*-559 (Metz *et al.* 1983, Widger *et al.* 1984). First information on its amino acid composition and partial N-terminal sequence (first 9 amino acid residues) has been obtained with the protein isolated and purified from spinach PS2 particles by Farchaus and Dilley (1986). These authors provided evidence for the unique character of the protein clearly distinct from the cytochrome *b*-559 and ATP synthase subunits. Later, Hird *et al.* (1991) determined the whole sequence of the protein by sequencing a region of wheat chloroplast DNA. Genes similar to that found in wheat have been also found in liverwort, tobacco, and rice (Ohyama *et al.* 1986, Shinozaki *et al.* 1986, Hiratsuka *et al.* 1989). Protein with a similar amino acid sequence has been also partially sequenced in *Chlamydomonas* (Dedner *et al.* 1988) and in the thermophilic cyanobacterium *Synechococcus vulcanus* (Koike *et al.* 1989). Gene encoding the PsbH protein from the cyanobacterium *Synechocystis* PCC 6803 has been cloned and sequenced by Abdel-Mawgood and

Received 25 November 2002, *accepted* 17 February 2003.
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Abbreviations: Chl – chlorophyll; DMBQ – 2,5-dimethyl-*p*-benzoquinone; IC7 – *psb*H-deletion mutant of the cyanobacterium *Synechocystis PCC* 6803; PS1 and PS2 – photosystem 1 and photosystem 2; WT – wild-type strain of the cyanobacterium *Synechocystis PCC* 6803.

Acknowledgment: We thank Prof. J. Barber for providing the *psb*H deletion mutant of *Synechocystis PCC* 6803. This work was sponsored by project MSM 12310001 of the Ministery of Education, Youth, and Sports of the Czech Republic and by Institutional Research Concept no. AV0Z5020903.

Table 1. Aligned N-terminal sequences of psbH homologs in various organisms (according to SWISS PROT database, May 2002). S.P.a.n., SWISS PROT accession number; organisms *1-8* do not contain LHC2-type of the PS2 antenna, organisms *9-23* contain LHC2-type of the external PS2 antenna. Tentatively phosphorylable threonine residues are in bold. * Sequence determined from the direct N-terminal protein sequencing.

Organism	S.P.a.n.	Aligned N-terminal sequence
1 Prochlorothrix	P31095	----------GQKTALSNFLKPFNSNAGKVVPG
2 Cyanidium	O19925	----------ALKTRLGELLRPLNSQYGKVAPG
3 Cyanophora	P48105	----------PQRTALGNILRPLNSEYGKVAPG
4 Guillardia	O78514	----------ALRTRLGELLRPLNSEYGKVAPG
5 Odontella	P49475	----------ALRTRLGEI LRPLNAEYGKVAPG
6 Porphyra	P51325	----------ALRTRLGEILRPLNSEYGKVAPG
*7 Synechococcus**	P19052	----------ARRTWLGDILRPLNSEYGKV
8 Synechocystis	P14835	----------AQRTRLGDILRPLNSEYGKVVPG
9 Chlamydomonas	P22666	ATGT—SKAKPSKVNSDFQEPGLVTPLGTLLRPLNSEAGKVLPG
10 Chlorella	P56323	ATGTTSKVKVS----------GVSTPLGTLLKPLNSEYGKVAPG
11 Euglena	P31555	---**T**—TISKNKTSNSK----------GKTTTLGTI LKPLNSKYGKVLPG
12 Marchantia	P12160	ATQIIDDTPKTK----------GKKSGIGDI LKPLNSEYGKVAPG
13 Mesostigma	Q9MUV4	AD—**T**SQ----------GKRTVVGNFLKPLNSEYGKVAPG
14 Arabidopsis	P56780	ATQTVEDSSRSG----------PRSTTVGKLLFPLNSEYGKVAPG
15 Hordeum	P12363	ATQTVEDSSKPR----------PKRTGAGSLLKPL NSEYGKVAPG
16 Nicotiana	P06415	ATQTVENSSRSG----------PRRTAVGDLLKPLNSEYGKVAPG
17 Oenothera	P19820	ATQTAEESSRAR----------PKKTGLGGLLKPLNSEYGKVAPG
18 Oryza	P09449	ATQTVEDSSRPG----------PRQTRVGNLLKPLNSEYGKVAPG
19 Pinus	P41627	ATQTIDDTSKTT----------PKETLVGTTLKPLNSEYGKVAPG
20 Populus	Q36632	ATQSVEGSSRSG----------PRRTIVGDLLKPLNSEYGKVAPG
21 Spinacea	P05146	ATQTVESSSRSR----------PKPTTVGALLKPLNSKYGKVAPR
22 Triticum	P04965	ATQTVEDSSKPR----------PKRTGAGSLLKPLNSEYGKVAPG
23 Zea	P24993	ATQTVEDSSRPK----------PKRTGAGSLLKPLNSEYGKVAPG

Dilley (1990) and Mayes and Barber (1991). From that time genes encoding PsbH homologues have been identified and completely sequenced in more than 25 organisms showing that the PsbH protein is a common component of PS2 in all oxygenic photosynthetic organisms (Table 1).

Localisation and expression of the gene in chloroplasts and cyanobacteria

In plants the *psb*H gene exhibits a conservative location in the *psb*B-*psb*H-*pet*B-*pet*D operon. The order and orientation of these genes are highly conserved in plant chloroplast genomes (*e.g.* Westhoff *et al.* 1983, Ohyama *et al.* 1986, Shinozaki *et al.* 1986). A range of transcripts of various sizes containing the entire operon or its components can be detected in chloroplasts (Westhoff and Herrmann 1988, Westoff *et al.* 1991). This means that all genes in the operon are co-transcribed and afterwards the primary transcript is processed. In *Synechocystis* PCC 6803, the *psb*H gene is a component of a different gene cluster containing *psb*N, *psb*H, *pet*C, and *pet*A genes (Mayes and Barber 1991). However, in contrast to the situation in chloroplasts, the *psb*H is transcribed as monocistronic mRNA while *pet*C-*pet*A genes as a separate, dicistronic unit (Mayes *et al.* 1993). In both types of organisms, the *psb*H gene is localised in a vicinity of genes coding for components of the cytochrome b_6f complex. Functional importance of this association is possible, although it is challenged by the fact that in *Chlamydomonas reinhardtii* the genes encoding components of the cytochrome b_6f complex are localised elsewhere in the chloroplast genome, and that the *psb*H gene exists in the cluster with *psb*B and *psb*T genes that code for CP47 and a small PS2 subunit PsbT, respectively. On the basis of analysis of nuclear mutants with impaired accumulation of *psb*B, *psb*T, and *psb*H transcripts it has been assumed that these three genes are co-transcribed (Monod *et al.* 1992, Johnson and Schmidt 1993). However, no interdependence of *psb*B/*psb*T and *psb*H mRNA accumulation suggests that *psb*B/*psb*T and *psb*H have separate promoters and each can be transcribed independently (Summer *et al.* 1997).

Despite the ongoing transcription of *psb*B and *psb*H genes, only *psb*H gene product could be identified in membranes of etiolated plant seedlings while CP47, the product of *psb*B, is not accumulated (Hird *et al.* 1991). Therefore, the transcription of the *psb*H gene and accumulation of its translation product is light independent.

Functional and structural role of the protein in PS2

Based on a certain similarity with the sequence of a LHC2 protein, Allen and Holmes (1986) proposed that the PsbH protein may bind Chl and play a role in the distribution of energy between both photosystems, as a part of the LHC2 complex. However, the absence of histidine residues in this single transmembrane helix-containing protein (Fig. 1) raised doubts about its ability to bind-Chl. The hypothesis was also questioned after the discovery of PsbH in cyanobacteria lacking LHC2. A further negation of the hypothesis was brought about by the finding that the protein is present in etioplasts while known Chl-binding proteins, including CP47, are stabile incorporated into the membrane only after irradiation when Chl is synthesised and stabilises the apoproteins (Eichacker *et al.* 1990). Based on the correlation between the phosphorylation of the PS2 proteins (Hodges *et al.* 1985), particularly of the PsbH protein (Packham 1987), and the electron transfer rate from the primary to the secondary quinone acceptors Q_A and Q_B, it has been proposed that the re-oxidation rate of Q_A is dependent on the phosphorylation status of the PsbH protein. This could indicate a close proximity of the PsbH protein to the reaction centre of PS2. First more extensive characterisation of the protein role in PS2 has been allowed by the construction of the *psb*H-less mutant of *Synechocystis PCC* 6803 (Mayes *et al.* 1993). The PS2 complex of the mutant differed from the WT complex in several aspects. Compared to the WT, the mutant strain was able to grow autotrophically but more slowly and only under low irradiance. In the absence of the PsbH protein, PS2 remained functional, but electron transfer between Q_A and Q_B was significantly slower, as revealed by fluorescence measurements (Mayes *et al.* 1993; Fig. 2). Measurements of thermoluminescence and flash-induced oxygen evolution revealed modification of the Q_B binding site but the influence of the PsbH absence on the donor side of PS2 and on the oxygen evolving complex could not be excluded. Recently, a more extensive characterisation of the *psb*H deletion mutant revealed further features of the PsbH-deficient PS2 (Komenda *et al.* 2002). We have found that after removal of CO_2 from the medium, electron transport between Q_A and Q_B is further slowed down. However, this change can be easily reversed by the addition of bicarbonate. In contrast, in the wild type strain (WT) the rate of Q_A re-oxidation remains the same after CO_2 removal. Also the very fast inactivation of DMBQ-supported oxygen evolving activity under high irradiance, typical for the mutant (Komenda and Barber 1995), can be either accelerated by CO_2 removal or slowed down on the addition of bicarbonate immediately prior to irradiation. This result corroborates the results of Sundby *et al.* (1989) who showed a negative correlation between the phosphorylation of the PsbH protein and the concentration of bicarbonate in plant thylakoids. These data indicate that the PsbH protein is needed for the stable binding of bicarbonate on the PS2 acceptor side.

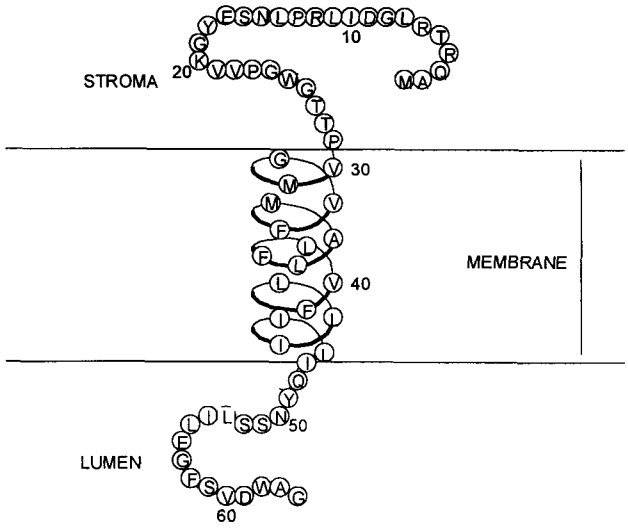

Fig. 1. Putative structure of the PsbH protein from the cyanobacterium *Synechocystis* PCC 6803.

After exposure of the mutant cells to high irradiance (1 000 μmol m^{-2} s^{-1}) formation of the D1-cytochrome *b*-559 adduct and formation of the D1 fragments has been identified (Komenda *et al.* 2002). At the same time, the oxygen-dependent shift of the D1 protein mobility in the electrophoretic gel and positive identification of the D1 band by an *Oxyblot* kit showed that increased oxidation of the D1 protein occurs in the mutant. As these features were unique for the mutant, and were not observed in the WT strain, it is assumed that in the absence of the PsbH protein, the impaired function of PS2 leads to increased probability of the formation of reactive oxygen species. These oxygen species oxidise the D1 protein which can be subsequently cross-linked with the α-subunit of cytochrome *b*-559 or fragmented.

A very important feature of the protein is its susceptibility to phosphorylation. This post-translational modification has been demonstrated in higher plants (Bennett 1977) and in the green alga *Chlamydomonas* (Dedner *et al.* 1988). The protein seems to be phosphorylated on two residues, one was identified as threonine 2 (Michel and Bennett 1987, Michel *et al.* 1988) while the other remains unknown (Vener *et al.* 2001). In line with this, a double band of phosphorylated PsbH in the PS2 of green algae *Chlamydomonas* and *Chlorella* has been identified with anti-phosphothreonine antibody (Hamel *et al.* 2000, Komenda *et al.* 2002, and Fig. 3). There is also a report on PsbH phosphorylation in *Synechocystis* (Race and Gounaris 1993) despite the absence of threonine in the

N-terminus segment of the protein in this organism. A recent Western blot analysis of threonine protein phosphoryla-tion in thylakoids and PS2 complexes in *Synechocystis* did not reveal any phosphoproteins (Fig. 3) and also la-belling with ^{33}P did not provide any evidence for the phosphorylation of the PsbH protein (Komenda *et al.* 2002). The aligned N-terminal sequences of PsbH (Table 1) show that all organisms not-containing LHC2-type of the PS2 antenna (prochlorophytes, cyanobacteria, rhodo-phytes, and diatoms) contain the PsbH protein with shor-ter N-terminal part without the phosphorylable threonine. Therefore, we believe that the phosphorylation of PsbH is important for the function of PS2 in the LHC2-containing stacked regions of the membrane. In line with this Giardi *et al.* (1994) showed that de-phosphorylation of PsbH by alkaline phosphatase causes an extremely fast inactivation of the PS2 in isolated spinach thylakoids, while in cyano-bacterial membranes the enzyme does not affect PS2 electron transfer activity.

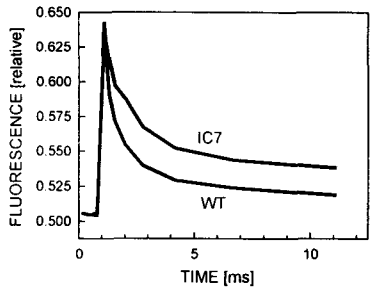

Fig. 2. Re-oxidation of the PS2 primary electron acceptor Q_A is slowed down in the absence of the PsbH protein. Re-oxidation of the PS2 primary electron acceptor Q_A in cells of the WT strain (*solid line*) and the psbH-deletion mutant IC7 (*dotted line*) was measured by the double modulated fluorimeter *P.S.I.* as decay of the variable component of fluorescence elicited by strong flash after 5-min dark adaptation.

Phosphorylation of the PsbH increases with irradiance in chloroplasts (Ebbert and Godde 1994) and also in plant leaves (Rintamäkki *et al.* 1997). In isolated plant thylakoids the phosphorylated PsbH is steadily accumulated during 60-min irradiation and no saturation has been observed (Štys *et al.* 1995). It is in contrast with phosphorylation of LHC2 that has its maximum at ambient irradiance but becomes inhibited at higher irradiance or after 30 min irradiation.

Besides its role in the function of the PS2 core complex, the PsbH protein is important also for the biogenesis and the structure of PS2. Deletion of the protein in *Chlamydomonas reinhardtii* leads to the disappearance of PS2 from the thylakoid membrane, documenting its role in the stable assembly of PS2 (Summer *et al.* 1997, O'Connor *et al.* 1998). Analysis of the PS2 assembly intermediates by radioactive pulse labelling showed that the synthesis of main protein components of the PS2 core D1, D2, CP43, and CP47 is not affected but their assembly into larger PS2 complexes is inhibited (Summer *et al.* 1997). In the PsbH-deletion mutant of *Synechocystis* the turnover

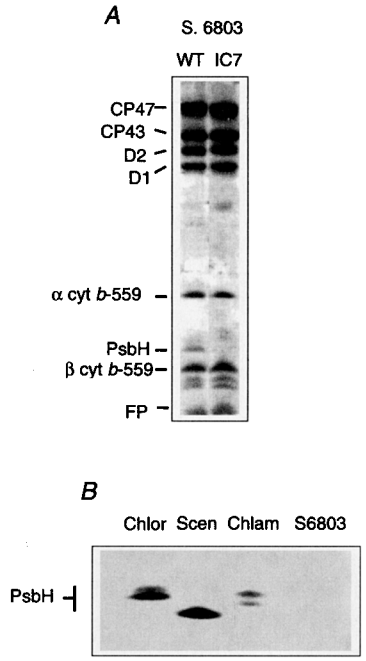

Fig. 3. (*A*) Identification of the PsbH protein in *Synechocystis* PS2 core complexes of WT and psbH deletion mutant IC7 and (*B*) its phosphorylation in PS2 core complexes of green algae *Chlorella* (Chlor), *Scenedesmus* (Scen), and *Chlamydomonas* (Chlam), and of the cyanobacterium *Synechocystis* (S6803). PS2 core complexes were obtained by *Deriphat*-PAGE of dodecylmaltoside solubilised thylakoids. Analysis was performed on 12-20 % polyacrylamide gel containing 7 M urea, gel was either stained by Coomassie Blue (*A*) or electroblotted, and phosphoproteins on the nitrocellulose membrane were detected by anti phosphothreonine antibodies (*Zymed*, USA; *B*).

of the D1 protein is slowed down and recovery from photoinhibition is also slower (Komenda and Barber 1995). This can be related to the effect described above, as the restoration of the PS2 activity occurs after the D1 replacement during which at least partial reassembly or rearrangement of PS2 subunits is required. As shown in the deletion mutant of *Chlamydomonas*, this process is exactly what is inhibited. A role of the PsbH protein in the stabilisation of PS2 has also been supported by our recent experimental data. We have isolated the PS2 core complex from the deletion mutant of *Synechocystis* and subjected it to *Deriphat* non-denaturing electrophoresis. In contrast to the isolated PS2 core from WT, a large amount of the reaction centre complex D1-D2-cytochrome *b*-559 appeared on the gel indicating that PsbH protein may stabilise the binding of CP47 to the D1-D2 heterodimer (Komenda *et al.* 2002). Such a role could also explain the instability of the PS2 core in *Chlamydo-*

monas, since weak binding of CP47 to the heterodimer could allow a fast proteolysis of PS2 subunits before the complex becomes fully assembled.

Recently, Zouni *et al.* (2001) published a model of PS2 of the thermophilic cyanobacterium *Synechococcus elongatus*, based on X-ray structural analysis at 35 nm resolution. In this model, the helix on the side of CP47 and D2, in the proximity of the Q_A-Q_B region, was tentatively ascribed to the PsbH protein. This position of the PsbH protein was chosen because of the apparent effect of PsbH phosphorylation (in higher plants) or the PsbH absence (in cyanobacteria) on the electron transfer between the PS2 quinone acceptors. This localisation is in agreement with our data since it can also explain the recently observed stabilisation effect on the binding of CP47 to the D1-D2 heterodimer (Komenda *et al.* 2002). Using Blue-Native Gel Electrophoresis, a mild electrophoretic method for separation of native membrane complexes according to their size, no PS2 dimers were identified in the *psb*H-deletion strain IC7 of *Synechocystis* (Fig. 4). This is again in agreement with position of the protein in the interface between the PS2 monomers. The proximity of the PsbH protein to the PS2 reaction centre is also suggested by results of Kuhn *et al.* (1988) and Chiaramonte *et al.* (1999). A parallel degradation of the D1 and the PsbH proteins and their tentative cross-linking during irradiation of isolated spinach thylakoids was found (Kuhn *et al.* 1988). Chiaramonte *et al.* (1999) suggested close structural interaction of the D1 and PsbH subunits in PS2 from *Synechocystis* due to the observed conformational modification of the Q_B herbicide binding pocket that is caused by the replacement of the native PsbH by the homologue from maize (see also Bergantino *et al.* 2002). These data taken together are not in contradiction with the hypothesis of Packham (1988) who considered the PsbH protein as a possible functional homologue of the H subunit in the reaction centre of photosynthetic bacteria.

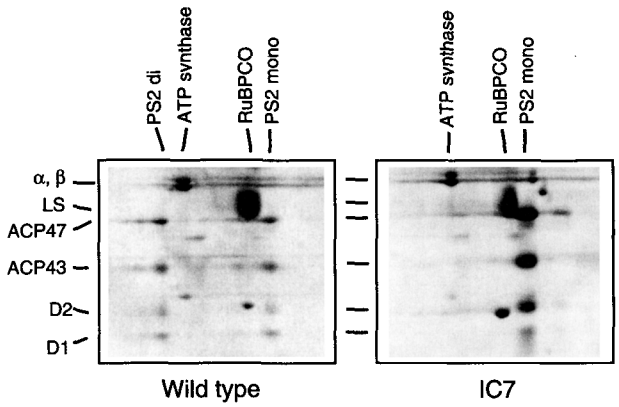

Fig. 4. The PS2 dimer is absent in the thylakoids of the *Synechocystis psb*H deletion mutant IC7. Thylakoids from the wild type (*left panel*) and IC7 mutant (*right panel*) were solubilised by dodecylmaltoside and complexes were analysed by Blue Native PAGE. Subunit composition of the complexes was determined by SDS-PAGE on 12-20 % acrylamide gel containing 7 M urea, and the 2-D gel was stained by Coomassie Blue. LS – large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO); α, β – subunits of ATP synthase; PS2 di and mono – dimer and monomer of the PS2 core complex.

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