S8-003

Identification of possible assembly and repair factors in photosystem two preparations of *Synechocystis* sp. PCC 6803: A new model for D1 turnover.

P J N Silva and P J Nixon

Wolfson laboratories, Department of Biological Sciences, Imperial College of Science Technology and Medicine, London SW7 2AY, UK. E-mail: paulo.silva@ic.ac.uk Fax: +442075945267

Keywords: photoinhibition, slr0228, D1 turnover, FtsH, Phb1

Introduction

Upon exposure to high light, oxygenic photosynthetic organisms, including cyanobacteria, suffer from a reduction in photosynthetic activity termed photoinhibition. The major target for light-induced damage is the D1 polypeptide of the multi-subunit photosystem two complex (PSII). Under light stress an impairment of electron transport (photoinactivation) and irreversible damage to reaction centre subunits (mainly to the Dl protein) occurs (reviewed in Barber and Andersson, 1992; Ohad *et al*., 1994). Plants and oxygenic organisms overcome this damage by making use of a repair process, which involves the degradation of damaged D1 and its replacement by a newly synthesised copy, possibly in a synchronised process (Komenda and Barber, 1995; Komenda *et al*., 1999; Komenda and Masojidek, 1995). The molecular details underpinning this repair process remain unclear.

The FtsH family of proteases are found throughout nature and are implicated in the degradation of both integral and soluble proteins (Karata *et al*., 1999) including secondary breakdown products of D1 in chloroplasts (Spetea *et al.*, 1999; Lindahl *et al.,* 2000) Recently we discovered that D1 turnover *in vivo* was severely reduced in a mutant of *Synechocystis* sp. PCC 6803 (slr0228::Ω) in which one of the four *ftsH* genes (slr0228) had been disrupted (Silva, Bailey, Mullineaux, Robinson, Mann, Nixon, unpublished work). Mutation of its closest homologue, *VAR2*, in *A. thaliana* also reduces the rate of D1 turnover (Bailey *et al.*, 2001). Our data on slr0228 and VAR2 is therefore consistent with a role for these FtsH homologues in D1 degradation. However, the effects observed *in vivo* on D1 turnover may be direct, indirect or a combination of both.

In this report we show that FtsH is present in PSII preparations isolated from a mutant of *Synechocystis* sp. PCC 6803 in which the PsbB subunit (CP47) of PSII is His-tagged (Bricker *et al*., 1998). FtsH thus appears to be able to bind to PSII. In addition we also show that this type of preparation also contains prohibitin (Phb1), a protein previously found in mitochondria and thought to be involved in the assembly of electron transfer complexes (Nijtmans *et al.*, 2000).

Based on our results and recent studies on the biogenesis of protein complexes in mitochondria (Steglich *et al.*, 1999), we propose a new model for the repair of PSII that emphasises a key role for FtsH and Phb1.

Materials and methods

The glucose-tolerant strain of *Synechocystis* sp. PCC 6803 (Williams, 1988) and mutant HT-3 containing a C-terminal histidine-tag on CP47 (Bricker *et al*., 1998) were grown in BG11 containing 5mM glucose. All cultures were grown at a light intensity of $30\mu Em^{-2}s^{-1}$ and harvested when A_{730} was between 0.7-0.9. Thylakoids were isolated and solubilised according to Tang and Diner (1994). The soluble fraction was subsequently subjected to Ni^{2+}-affinity chromatography. Several washings were performed and typically the wash before the imidazole elutions in batch mode presented an A_{674} between 0.05-0.07. The elution with 100mM imidazole always gave an absorbance above 2. SDS-PAGE gels were run overnight in a LKB 2001 vertical electrophoresis apparatus. Running buffer was a variation of the Laemmli buffer, with a pH of 8.3 (25mM Tris, 190mM Glycine and 0.1% (w/v) SDS) (Laemmli, 1970). Immunodetection was performed using ECL chemiluminescence method (Amersham) following the manufacturer's instructions.

Results

To test if FtsH could interact directly with PSII, we decided to investigate whether FtsH could be detected immunochemically in His-tagged PSII preparations of *Synechocystis* sp. PCC 6803 HT-3. We reasoned that such a preparation might include not only fully functional PSII, but also complexes that were in the process of being assembled or degraded.

Figure1A shows the analysis of His-tagged PSII eluted from a Ni^{2+}-column using increasing concentrations of imidazole. The bulk of PSII was eluted in the 100mM imidazole fraction as judged by coomassie-blue staining and the D1 immunoblot. The room-temperature absorbance spectra of the eluted fractions further confirmed the purity of the preparation (data not shown). As a control, a sample from the wild type control (lacking the His-tag) was chromatographed under identical conditions. In the absence of the His-tag, no PSII was retained by the Ni^{2+}-column (Fig 1B). Importantly FtsH could be detected immunochemically in the His-tagged PSII fractions. The presence of two immunoreactive bands is consistent with the presence of more than one of the FtsH homologues in this preparation. As FtsH is a Zn^{2+}-activated protease, it was vital to show that FtsH did not bind directly to the Ni^{2+}-column. Fig 1B confirms that FtsH in the WT control did not bind to the Ni^{2+}-column. In contrast, HtrA detected using an antiserum raised against mature HtrA (absence of signal sequence), was bound to the Ni^{2+}-column both in the WT control and the His-tagged PSII sample. The presence of HtrA in the His-tagged PSII preparation is therefore unlikely to be due to an interaction with PSII. In further experiments, FtsH was found to co-elute with His-tagged PSII in the presence of 500mM NaCl suggesting that the interaction between PSII and FtsH is not due to non-specific ionic interactions (data not shown).

In mitochondria, FtsH forms a supercomplex with a large prohibitin complex composed of two subunits Phb1 and Phb2. Analysis of the genome sequence of *Synechocystis* sp. PCC 6803 predicts that there are two prohibitin homologues designated slr1106 (Phb1) and slr1768 (Phb2). Using antiserum generated against *Synechocystis* sp. PCC 6803 Phb1, following over-expression in *E. coli*, we found that Phb1 was also present in the His-tagged PSII preparation.

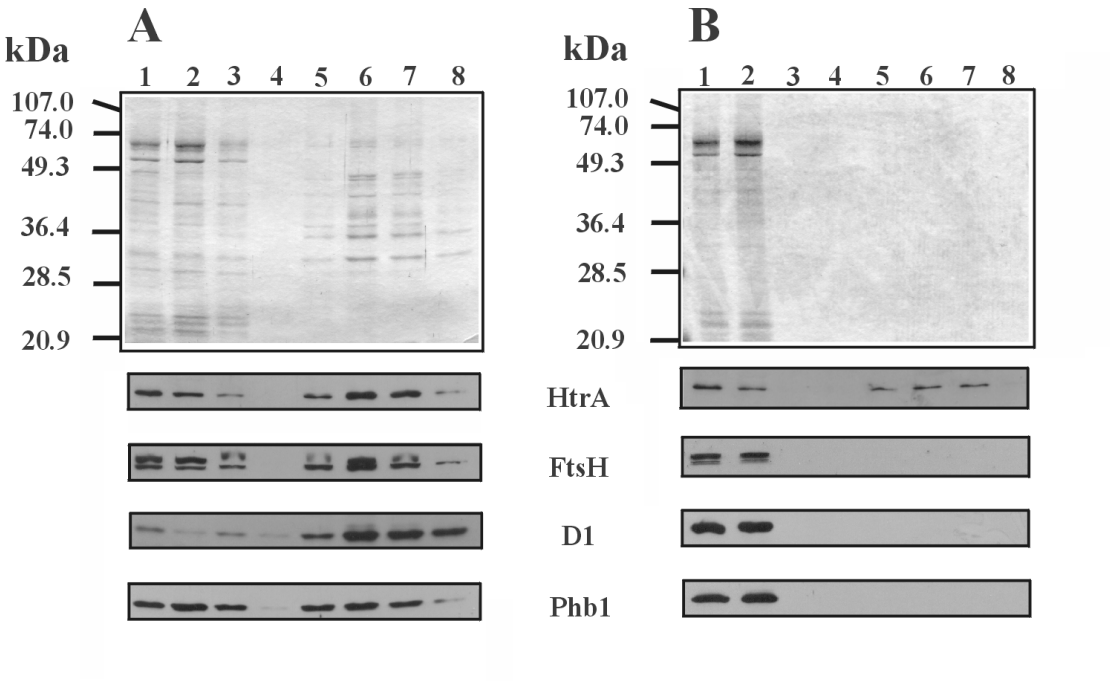

Fig. 1. Western analysis. (A) Coomassie-Blue stained 12% SDS-PAGE gel, containing 6M urea and several different immunoblots in *Synechocystis* sp. PCC 6803 HT-3; (B) Coomassie Blue-stained 12% SDS-PAGE gel, containing 6M urea and several different immunoblots in *Synechocystis* sp. PCC 6803-G. Lane 1-thylakoids, lane 2-unbound, lane 3-5th wash, lane 4-last wash, lane 5-50mM imidazole, lane 6-100mM imidazole, lane 7-150mM imidazole, lane 8-300mM imidazole. Lanes 1 and 2 contained 1µg of Chl, lanes 3-8 contained the same amount of eluted (40 µl). Relative migration of molecular weight standards is indicated on the left.

Discussion

We have shown that FtsH and Phb1 can be detected in His-tagged PSII preparations of *Synechocystis* sp. PCC 6803 HT-3, isolated using immobilised Ni^{2+}-affinity chromatography. Control experiments rule out the binding of FtsH and Phb1 to the Ni^{2+}-column. The presence of FtsH and Phb1 in the His-tagged preparation thus implicates their binding to CP47 and probably PSII. We speculate that the His-tagged PSII preparation contains in addition to active PSII a small proportion of complexes that bind FtsH and Phb1. These complexes may be repair or assembly complexes involved in the PSII repair cycle. Based on our studies on FtsH and the predicted roles of FtsH and Phb1 in the mitochondrion, we propose a new model for the D1 repair cycle (Fig. 2).

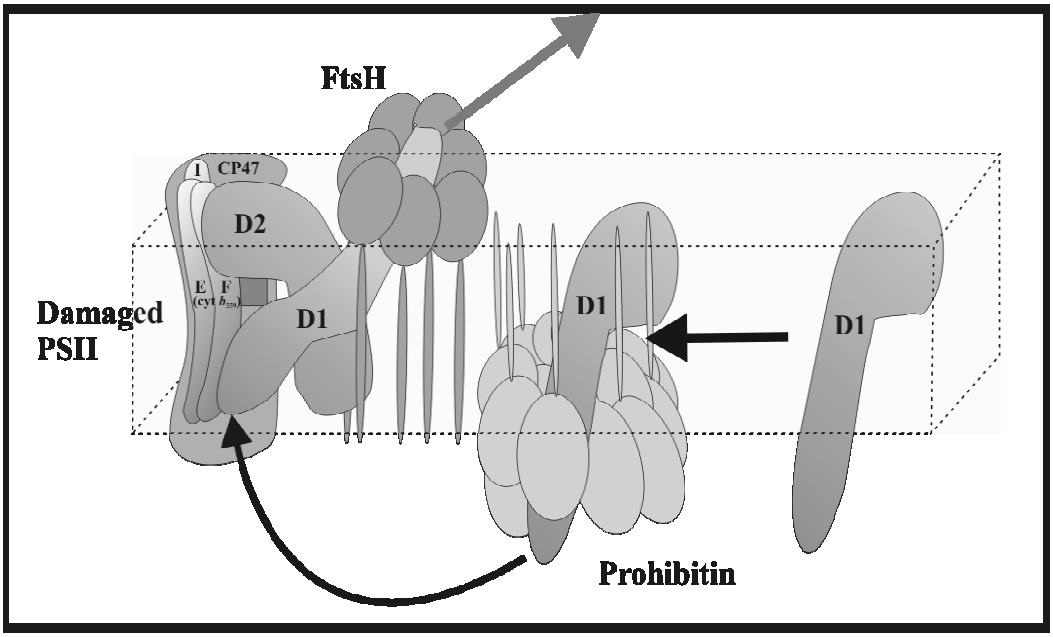

Fig. 2. A working model for the D1 repair cycle.

In mitochondria, there is evidence that the large Phb1/Phb2 complex assists in folding or holding newly synthesised subunits within the membrane prior to incorporation into a complex (Nijtmans *et al.*, 2000). Also a large FtsH complex, composed of two types of FtsH subunit, catalyses the removal of misfolded proteins and can form a supercomplex with Phb (Steglich *et al.*, 1999). We suggest that in *Synechocystis* and also in chloroplasts, a supercomplex of Phb1 and FtsH may catalyse the synchronised replacement of damaged D1 (and possibly other thylakoid membrane proteins). In this model the prohibitin complex prevents the unwanted degradation of newly-synthesised unassembled D1 in the membrane and the FtsH complex is involved in the highly processive ATP-driven degradation of D1 either by a 'pulling' or by a 'shedding' mechanism (Langer, 2000). Unlike previous models for D1 degradation, our analysis of the slr0228 null mutant indicates that FtsH is involved in the early steps of D1 degradation not just the removal of secondary breakdown products, whose formation may in fact be the result of relatively rare cleavage events. The presence of FtsH and Phb1 in cyanobacteria, mitochondria and possibly chloroplasts, also indicates that there may be an evolutionary conserved pathway for the removal of unassembled or damaged membrane proteins in organelles and bacteria. This does not, however, exclude the presence of other pathways for the repair of PSII. For instance DegP2, for which there is no obvious homologue in *Synechocystis* sp. PCC 6803, may also play a role in D1 degradation in the chloroplast (Haussuhl *et al.*, 2001).

Acknowledgments

We wish to thank Biotechnology and Biological Research Council and Fundação para a Ciência e Tecnologia for financial support. We also thank Professor T. Bricker for supplying us with the His-tagged *Synechocystis* sp. PCC 6803 mutant, Professor Teru Ogura for antibody anti FtsH, and Professor Mark Roberts for antibody anti HtrA.

References

Bailey, S, Silva, P, Nixon, P, Mullineaux, C, Robinson, C, Mann, N (2001). *Biochemical Society Transactions* **29**, 455-58.

Barber, J, Andersson, B (1992). *Trends Biochem Sci* **17,** 61-6.

Bricker, TM, Morvant, J, Masri, N, Sutton, HM, Frankel, LK (1998). *Biochim Biophys Acta* **1409,** 50-57.

Haussuhl, K, Andersson, B, Adamska, I (2001). *Embo J* **20,** 713-22.

Karata, K, Inagawa, T, Wilkinson, AJ, Tatsuta, T, Ogura, T (1999). *J Biol Chem* **274**, 26225-32.

Komenda, J, Barber, J (1995). *Biochemistry* **34,** 9625-31.

Komenda, J, Masojidek, J (1995). *Eur J Biochem* **233,** 677-82.

Komenda, J, Koblizek, M, Masojidek, J (1999). *J Photochem Photobiol* **48,** 114-119.

Langer, T (2000). *TIBS* **25,** 247-51.

Laemmli, UK (1970). *Nature* **227,** 680-85.

Lindahl, M, Spetea, C, Hundal, T, Oppenheim, AB, Adam, Z., Andersson, B (2000). *Plant Cell* **12,** 419-31

Nijtmans, LGJ, de Jong, L, Artal Sanz, M, Coates, PJ, Berden, JA
Back, JW, Muijsers, AO, van der Spek, H, Grivell,LA(2000). *EMBO J* **19**, 2444-51.

Ohad, I, Keren, N, Zer, H, Gong, H, Mor, TS, Gal, A, Tal, S, Domovich, Y (1994) in *Photoinhibition of photosynthesis*, eds. Baker, NR, Bowyer, JR (Bios Scientific Publishers, Oxford), 161-77.

Spetea, C, Hundal, T, Lohmann, F, Andersson, B (1999). *Proc Natl Acad Sci U S A* **96,** 6547-52.

Steglich,G, Neupert,W, Langer,T (1999). *Mol Cell Biol* **19**, 3435–42.

Tang, XS, Diner, BA (1994). *Biochemistry* **33,** 4594-03.

Williams, JGK (1988) in *Methods in Enzymology*, eds. Glazer, A.N. & Packer, L. (Academic Press, San Diego), **167**, 766-78.