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# Multiple copies of the PsbQ protein in a cyanobacterial photosystem II assembly intermediate complex

Haijun Liu[1] · Daniel A. Weisz[1,2] · Himadri B. Pakrasi[1]

**Abstract** Photosystem II (PSII) undergoes frequent damage owing to the demanding electron transfer chemistry it performs. To sustain photosynthetic activity, damaged PSII undergoes a complex repair cycle consisting of many transient intermediate complexes. By purifying PSII from the cyanobacterium *Synechocystis* sp. PCC 6803 using a histidine-tag on the PsbQ protein, a lumenal extrinsic subunit, a novel PSII assembly intermediate was isolated in addition to the mature PSII complex. This new complex, which we refer to as PSII-Q4, contained four copies of the PsbQ protein per PSII monomer, instead of the expected one copy. In addition, PSII-Q4 lacked two other lumenal extrinsic proteins, PsbU and PsbV, which are present in the mature PSII complex. We suggest that PSII-Q4 is a late PSII assembly intermediate that is formed just before the binding of PsbU and PsbV, and we incorporate these results into an updated model of PSII assembly.

**Keywords** Photosynthesis · Photosystem II assembly · Membrane protein · Thylakoid membrane

Haijun Liu and Daniel A. Weisz have contributed equally to this study.

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## Introduction

Photosystem II (PSII) is a large membrane bound pigment-protein complex that uses light to drive reduction of plastoquinone, while oxidizing water to molecular oxygen. PSII is found in all oxygenic photosynthetic organisms, including cyanobacteria, algae, and higher plants, and its composition and function have been largely conserved across species and evolutionary time (Vinyard et al. 2013). Active PSII is generally found as a mixture of monomers and dimers, with dimers being the major and more-active species (Rögner et al. 1987; Watanabe et al. 2009; Nixon et al. 2010; Nickelsen and Rengstl 2013). Based on the available crystal structures of PSII and biochemical evidence, each monomer contains 20 proteins, as well as numerous protein-bound cofactors, including 35 Chlorophyll *a* (Chl *a*) molecules, two pheophytins, two plastoquinones, 11 β-carotenes, two hemes and one non-heme iron, four manganese ions, one calcium, and one chloride ion (Roose et al. 2007a, b; Suga et al. 2015; Umena et al. 2011). The D1 and D2 proteins primarily coordinate the major cofactors involved in PSII photochemistry, including P680, the chlorophyll pair whose light-driven oxidation provides the driving force for water oxidation (Vinyard et al. 2013; Rappaport and Diner 2008). CP43 and CP47 are the two core chlorophyll-binding antenna proteins and funnel excitation energy to P680 to initiate the reaction (Bricker and Frankel 2002). Twelve low-molecular-weight membrane-spanning proteins associate with PSII as well, each of which appears to have a role in optimizing PSII structure and activity under various environmental conditions, and/ or in assisting in PSII assembly and repair (Shi and Schröder 2004; Shi et al. 2012). Water oxidation is accomplished via a $Mn_4CaO_5$ cluster, also called the water oxidation complex (WOC), that is coordinated by D1 and

CP43 near the lumenal side of the membrane (Vinyard et al. 2013), and whose structure has been determined recently to near-atomic resolution (Umena et al. 2011). Three extrinsic, soluble proteins, PsbO, PsbU, and PsbV, bind to the lumenal side of cyanobacterial and red algal PSII, helping stabilize the WOC and allowing the cell to maintain high rates of oxygen evolution (Roose et al. 2007a, b; Bricker et al. 2012). Green algae and higher plants also contain PsbO, but lack PsbU and PsbV. They instead contain PsbP, PsbQ, and PsbR, which appear to serve as their functional replacements (Bricker et al. 2012).

Although it is not observed in the available PSII crystal structures from either *Thermosynechococcus elongatus* BP-1 or *Thermosynechococcus vulcanus* (Suga et al. 2015; Umena et al. 2011; Zouni et al. 2001; Kamiya and Shen 2003; Ferreira et al. 2004; Loll et al. 2005; Guskov et al. 2009), experimental evidence indicates that active cyanobacterial PSII contains a fourth extrinsic protein, PsbQ (Roose et al. 2007a, b, Kashino et al. 2002; Thornton et al. 2004). Recently, we used chemical cross-linking and mass spectrometry to demonstrate that PsbQ binds to the lumenal side of PSII, and interacts closely with both PsbO and CP47 (Liu et al. 2014). This study also detected a close interaction between copies of PsbQ present in two different monomers. This result indicates that PsbQ is located near the PSII dimer interface, and suggests that a PsbQ–PsbQ interaction may help stabilize the PSII dimer.

Given its size and structural complexity, assembly of PSII is a highly ordered and tightly regulated process (Nickelsen and Rengstl 2013; Komenda et al. 2012). The first major assembly intermediate formed is the reaction center (RC) complex, consisting of the D1, D2, PsbE, PsbF, and PsbI proteins (Nickelsen and Rengstl 2013; Komenda et al. 2004). Binding of CP47 and several low-molecular-mass proteins to RC leads to the formation of the RC47 complex (Boehm et al. 2012). CP43 and several other low-molecular-mass proteins then bind to RC47 (Nixon et al. 2010). After C-terminal processing of the D1 protein and dissociation of Psb27 from the lumenal side of CP43, the manganese cluster is able to assemble at the lumenal interface of D1 and CP43 (Roose and Pakrasi 2008; Grasse et al. 2011; Liu et al. 2011, 2013). The extrinsic proteins are able to bind at this stage, stabilizing the WOC (Komenda et al. 2012; Liu et al. 2011). Photoactivation of the WOC produces a PSII complex capable of oxygen evolution (Dasgupta et al. 2008; Becker et al. 2011), which dimerizes to form the primary active PSII complex (Komenda et al. 2012; Nowaczyk et al. 2006). Thus, at any given instant in the cell, a small subset of PSII complexes are in various stages of assembly, in addition to the major subset of fully assembled, active complexes.

The D1 protein (and, to a lesser extent, other PSII proteins) incur frequent damage as a result of the high oxidizing potentials produced at $P680^+$ and other cofactors, and from the production of singlet oxygen that can result from certain unproductive charge recombination pathways during electron transfer (Aro and Allahverdiyeva 2012; Tyystjärvi 2013; Krieger-Liszkay et al. 2008; Adir et al. 2003). Such damage leads to photoinactivation of PSII, triggering a complex repair mechanism. After D1 is damaged, it is believed that PSII is partially disassembled (possibly to the RC47 stage), the damaged D1 is removed and degraded, a newly synthesized copy of D1 is inserted, and the full complex is reassembled (Nickelsen and Rengstl 2013; Nowaczyk et al. 2006). Significant progress has recently been made in understanding the PSII assembly and repair cycles (Nickelsen and Rengstl 2013; Nowaczyk et al. 2006). Nonetheless, the precise natures of many of the intermediate complexes still remain elusive.

In the present study, we have identified a PSII complex that contains four copies of PsbQ per PSII monomer. Based on its subunit composition, we propose that it is a late PSII assembly intermediate formed after the binding of PsbO, but before the binding of PsbU and PsbV.

## Experimental procedures

### Cyanobacterial culture and PSII purification

The HT3 strain of *Synechocystis* 6803 was a generous gift from Dr. Terry M. Bricker (Bricker et al. 1998). Generation of the Q-His strain of *Synechocystis* 6803 was previously reported (Roose et al. 2007a). Cyanobacterial strains were grown in BG11 medium. Purification of histidine-tagged PSII complexes was performed as described previously (Kashino et al. 2002) with minor modifications as follows: the first step of the two-step elution gradient was a linear increase from 0 to 50 mM histidine for 10 min at 0.5 mL/min, followed by a hold at 50 mM histidine for 5 min. The histidine concentration in the elution buffer was then switched to 200 mM histidine, and held at this concentration for an additional 23.5 min.

### 77 K fluorescence spectroscopy

Fluorescence emission spectra at 77 K were recorded on a Fluoromax-2 fluorometer (JobinYvon, Longjumeau, France) with excitation at 435 nm. PSII samples were diluted to 0.1-mg Chl *a*/mL in a buffer previously reported (Bricker et al. 1998). Fluorescence emission curves in Fig. 1b were normalized at 683 nm.
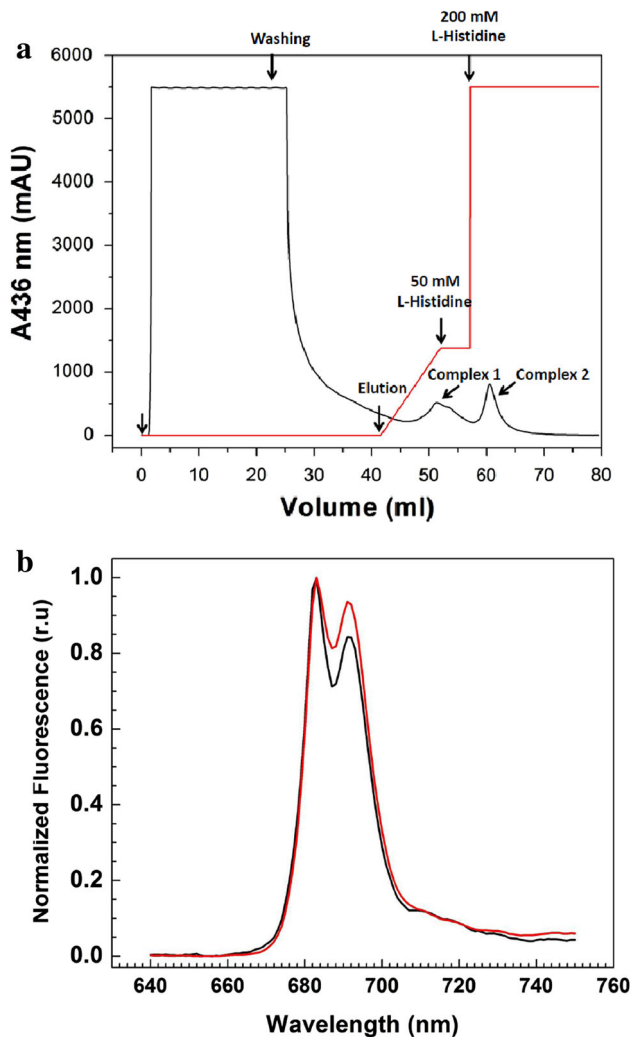
**Fig. 1** Isolation of two kinds of PSII complexes from the Q-His strain of *Synechocystis* 6803: **a** FPLC chromatogram for isolation of complex 1 and complex 2. The *first arrow* indicates the sample loading. Sample washing, first-step gradient elution and second-step gradient elution are also indicated; **b** fluorescence emission spectrum at 77 K for complex 1 (*red*) and complex 2 (*black*), with excitation at 435 nm. PSII peaks are located at 683 and 692 nm, respectively. Spectra were normalized at 683 nm

## Oxygen evolution measurements

The steady-state rate of oxygen evolution by PSII was measured on a Clark-type electrode at 5-µg Chl *a*/mL in 50-mM MES-NaOH (pH 6.5), 20-mM $CaCl_2$, 0.5-M sucrose, at 30 °C. Buffer contained 1-mM potassium ferricyanide and 0.5-mM 2,6-dichloro-*p*-benzoquinone as electron acceptors. Samples were incubated in the dark at 30 °C for 1 min before the onset of the measurement. Irradiance of 8250 µmol photons $m^{-2}$ $s^{-1}$ was used during the measurement.

## Protein gel electrophoresis and immunodetection

SDS-PAGE was performed as described previously (Kashino et al. 2001, 2002) unless otherwise indicated. After electrophoresis, proteins were transferred onto PVDF membranes (Millipore), and PSII proteins were probed using specific antisera. Bands were visualized with chemiluminescence reagents (West Pico; Pierce) on an ImageQuant LAS-4000 imager (GE Healthcare). Pixel densitometry analysis was performed using the Image-Quant TL software. Blue-native gel electrophoresis was performed as described previously (Schägger and von Jagow 1991). Silver staining of protein gels following SDS-PAGE was performed using metallic silver (Ag) protein stain according to the manufacturer's protocol (Thermo Scientific, Rockford, IL, USA).

## Results

PSII from the Q-His strain was purified by nickel-affinity chromatography using a two-step gradient of histidine-containing buffer (Fig. 1a, red line). The two elution peaks labeled in the chromatogram correspond to two distinct protein complexes, referred to as complex 1 and complex 2 (later identified as the PSII dimer (PSII-D) and PSII-Q4, respectively). They were collected separately and subjected to biochemical characterization. Complexes 1 and 2 represent approximately 53 and 47 % of the total PSII yield, respectively. In one of our previous studies in which PSII from the Q-His strain was purified, elution was performed with a single-linear gradient (Roose et al. 2007a), and the different elution conditions used in this study likely account for the isolation of a second PSII complex.

To determine if both elution peaks correspond to PSII complexes, 77 K fluorescence emission spectra were obtained. Both samples displayed characteristic "F685" (at 683 nm) and "F695" (at 692 nm) fluorescence signatures indicative of an assembled PSII reaction center (Fig. 1b) (Satoh 1980; Liu et al. 2011). The relative intensities of the two peaks were, however, slightly different, reflecting a slight structural difference between the two PSII complexes. A larger difference between the two complexes was observed by measuring their oxygen evolution activity; complex 2 evolved oxygen at only 66 % of the saturated rate of complex 1 (841 and 1270 µmol $O_2$·mgChl $a^{-1}$·$h^{-1}$, respectively).

SDS-PAGE analysis was performed to analyze the protein compositions of the two complexes. The resulting gel (Fig. 2a) shows that both complexes contained a nearly identical profile of the core PSII proteins compared to the control HT3-PSII sample (PSII from a strain with a His-tag
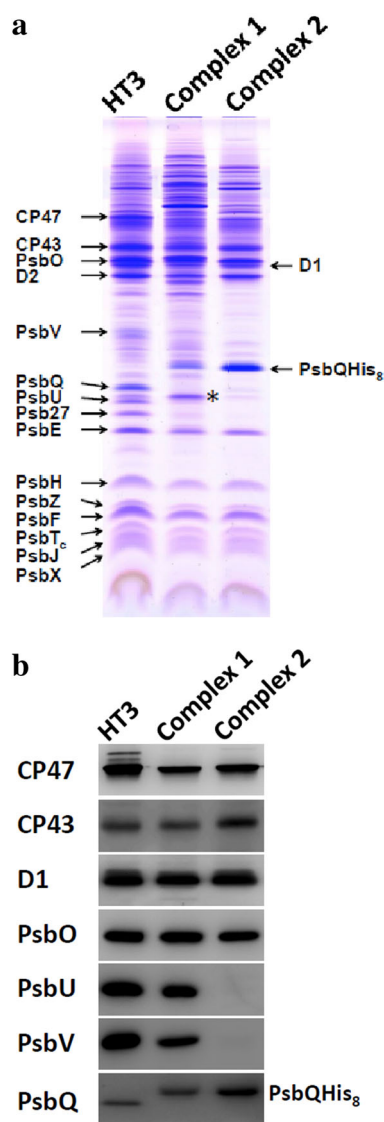
Fig. 2 Polypeptide compositions of complex 1 and complex 2: **a** SDS-PAGE protein profile with Coomassie Brilliant Blue staining. Each lane contained sample with 2.4 µg of Chl *a*. HT3, a PSII complex with a C-terminally polyhistidine-tagged version of CP47, is used as a reference. Major PSII subunits are labeled. PsbQHis8 is indicated on the *right*. Asterisk indicates a protein band containing Psb28 and Sll1130 encoded protein. Assignment of the PSII subunits is based on Kashino et al. (2002); **b** immunodetection of PSII polypeptides in the isolated complexes after SDS-PAGE. Each lane contained sample with 0.2 µg of Chl *a*. Specific antibodies against CP47, CP43, D1, PsbO, PsbU, PsbV, and PsbQ were used for immunodetection of the corresponding proteins

on the CP47 protein). However, Western blot analysis showed that complex 2 lacked the PsbU and PsbV proteins (Fig. 2b). In contrast, PsbO, the other lumenal extrinsic protein observed in the PSII crystal structure, was present in equal levels across all three complexes.

Surprisingly, the gel indicated that on a per-chlorophyll basis, complex 2 contained significantly more PsbQ than

complex 1. Immunoblot analysis of these three complexes using a PsbQ-specific antibody confirmed this result (Fig. 2b). The Western blot indicates that complex 1 and HT3-PSII contained roughly equal levels of PsbQ, implying that complex 2 contains more copies of PsbQ per PSII monomer than both complex 1 and HT3-PSII.

To determine if the additional copies of PsbQ in complex 2 were a contamination from free copies of the protein which are in fact unassociated with PSII, blue-native gel electrophoresis followed by denaturing gel electrophoresis and immunodetection was performed. The mild blue-native gel conditions (Schägger and von Jagow 1991) keep PSII complexes intact during electrophoresis, while unassociated proteins migrate separately due to their smaller size. Subsequent denaturing gel electrophoresis of individual excised native-gel bands allows characterization of the proteins that are present in a particular PSII complex. The native gel (Fig. 3a) showed that both complex 1 and complex 2 were present almost exclusively as dimers, consistent with our previous studies of Q-His-PSII (Liu et al. 2014). The dimer band was excised and analyzed by denaturing gel electrophoresis followed by silver staining (Fig. 3c, d), as well as by immunodetection (Fig. 3b, d). Both techniques confirmed the initial observation that PsbQ was present at an elevated level in complex 2 compared to complex 1. These results indicate that the additional copies of PsbQ found in complex 2 are indeed bound to the PSII complex, and are not unassociated-protein contaminants in the sample.

To quantify the increased level of PsbQ in complex 2, PsbQ content in a dilution series of complex 2 was detected by Western blot (Fig. 4a). Pixel densitometry analysis was performed to obtain a calibration curve of band intensity versus PsbQ content. By fitting PsbQ band intensity from complex 1 (from the same blot) to the calibration curve (Fig. 4b), we concluded that Complex 1 contains 23 % of the PsbQ content of complex 2, on a per chlorophyll basis; i.e. complex 2 contains four times as many copies of PsbQ as complex 1.

It is conceivable that the Q-His strain could produce a significantly higher quantity of PsbQ than the WT strain, potentially leading to an artifactual Q-His-PSII complex with higher PsbQ content. This possibility was ruled out by comparing PsbQ levels in the total membrane fraction of the Q-His and WT strains (Fig. 4c) by immunoblotting. After normalizing to D1 levels in the two strains, we determined that the PsbQ content of the Q-His strain was 1.1 ($\pm$0.3)-fold of that of the WT strain. This result indicates that the Q-His strain does not synthesize dramatically elevated levels of PsbQ compared to WT. Thus, the additional PsbQ content observed in complex 2 cannot be attributed to a change in the expression level of PsbQ in the Q-His strain.

**Fig. 3** Two-dimensional Blue-Native-PAGE and immunoblot analysis of complex 1 and complex 2: **a** Blue-native gel electrophoresis of HT3-PSII, complex 1, and complex 2. M, PSII monomer; D, PSII dimer; **b** Blue-Native-PAGE was followed by fractionation of complex 2 in the second dimension by SDS-PAGE, immunoblotting, and probing with anti-PsbQ antibody; **c** Blue-Native-PAGE was followed by fractionation of complex 1 and complex 2 dimers in the second dimension by SDS-PAGE and silver staining. *SSU* small protein subunits of PSII. Assignment of the distinct subunits of the Q-His protein complexes is based on Kashino et al. (2002), where we observed that the PsbF band also contains PsbI and PsbL, and the PsbX band also contains PsbM; **d** immunodetection of D1 and PsbQ in the BN-PAGE-isolated complex 1 dimer and complex 2 dimer. The silver-stained bands corresponding to PsbQ and PsbE are also shown
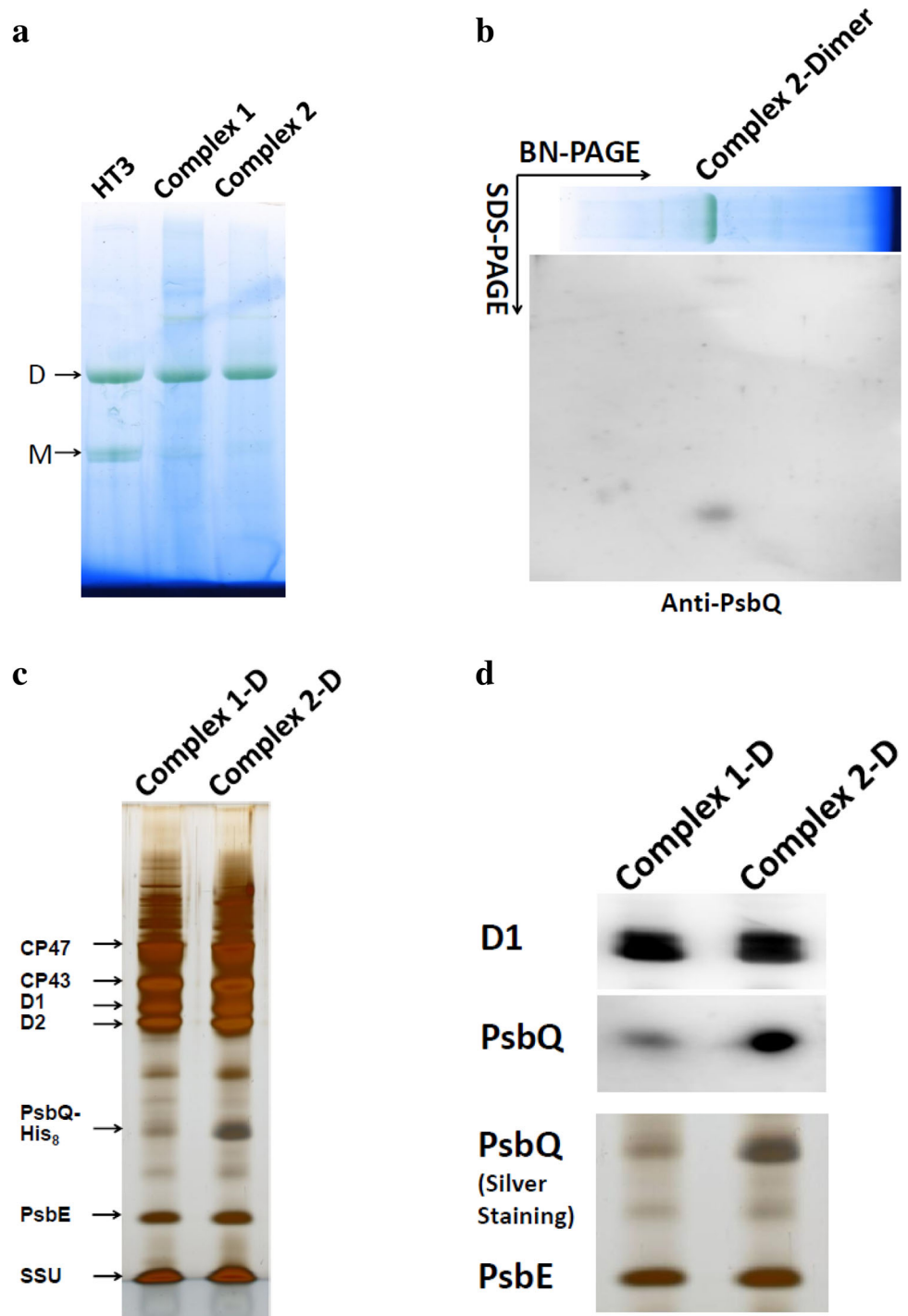


## Discussion

In this study, two types of PSII complex were isolated from the Q-His strain of *Synechocystis* 6803 by nickel-affinity chromatography using a stepped linear gradient of histidine during elution. Both complexes displayed the 77 K fluorescence signatures of an assembled PSII reaction center complex, and both were found almost exclusively as

dimers (Fig. 3a). Both contained a nearly identical profile of PSII proteins, compared to each other and to the control HT3-PSII complex. However, complex 2 contained four times as many copies of PsbQ as complex 1, and was lacking the extrinsic proteins PsbU and PsbV. The additional histidine tags in complex 2 caused increased affinity for the His-tag resin and explain its later elution time. Complex 2 exhibited 66 % of the oxygen evolution activity
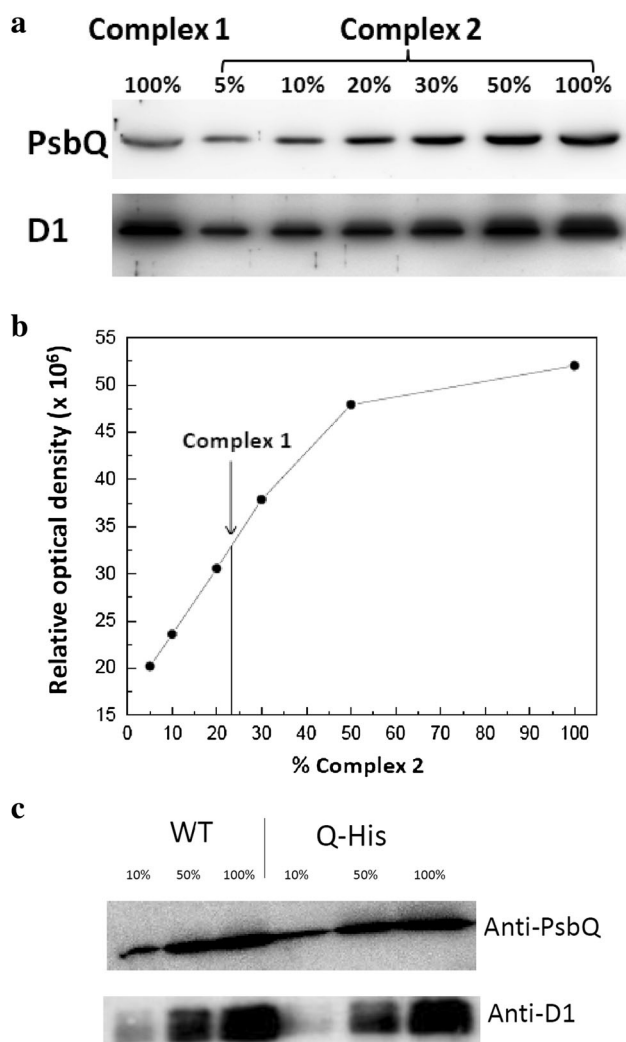
**a**



**b**



**c**



Fig. 4 Relative quantification of the PsbQ protein in complex 1 and complex 2: **a** SDS-PAGE followed by immunodetection, using anti-PsbQ and anti-D1 (as internal standard) antibodies, of a dilution series of complex 2 compared to a fixed quantity of complex 1. Loading ranged from sample containing 0.01-μg Chl *a* (5 %) to sample containing 0.2-μg Chl *a* (100 %). After immunodetection, pixel densitometry analysis of the PsbQ bands in complex 2 was used to construct the standard curve shown in **b**; the amount of PsbQ protein present in the complex 1 sample containing 0.2-μg Chl *a* was then determined from the standard curve. The *arrow* indicates the amount of PsbQ protein present in complex 1; **c** SDS-PAGE followed by immunodetection, using anti-PsbQ and anti-D1 (as internal standard) antibodies, of a dilution series of the total membrane fraction from WT and Q-His cells. Pixel densitometry analysis was performed as in **b** to quantify PsbQ content relative to D1 levels in the two strains (not shown)

of complex 1, which is understandable since PsbU and PsbV are known to optimize oxygen evolution capability (12–13). Based on these data, we conclude that complex 1 is the fully assembled active PSII dimer (PSII-D), and propose that complex 2 (hereafter PSII-Q4) is a newly identified PSII assembly intermediate.

Our earlier cross-linking studies have shown that fully assembled PSII contains one copy of PsbQ per PSII monomer (Liu et al. 2014). Hence, PSII-Q4 contains four copies. PsbU and PsbV are absent in PSII-Q4, and are roughly the same size as PsbQ (14.2, 17.9, and 15.7 kDa, respectively). Considering that all three proteins bind near each other on the lumenal surface of PSII (Umena et al. 2011; Liu et al. 2014), the extra copies of PsbQ in the PSII-Q4 assembly intermediate complex may temporarily occupy the binding regions of PsbU and PsbV. PsbQ contains a lipid modification that anchors it to the thylakoid membrane (Thornton et al. 2004; Kashino et al. 2006). This lipid anchor may position PsbQ near the lumenal surface of PSII, enabling facile, although relatively weak, binding of several copies of PsbQ to PSII during the assembly process, which are then replaced by PsbU and PsbV once they are able to diffuse to their binding sites.

The PsbQ deletion mutant in *Synechocystis* 6803 shows only a slight reduction in growth rate compared to the wild-type strain (Thornton et al. 2004; Summerfield et al. 2005) while the PsbV deletion mutant requires additional $Ca^{2+}$ and $Cl^-$ in the growth medium to enable photoautotrophic growth (Shen et al. 1998). However, the $\Delta psbV:\Delta psbQ$ double-deletion mutant is unable to grow photoautotrophically (Summerfield et al. 2005). It is possible that one or more copies of PsbQ in PSII-Q4 occupy the PsbV binding site, serving as an imperfect and temporary substitute for PsbV.

Enami and co-workers recently found that the PsbQ-type protein Psb31 is able to substitute partially for the role of PsbO in the centric diatom *Chaetoceros gracilis* (Nagao et al. 2010). In addition to Psb31, *C. gracilis* contains a second PsbQ-type protein, PsbQ′, whose lumenal binding site and role in optimizing oxygen evolution is distinct from those of Psb31 (Nagao et al. 2010; 2013). PsbQ′ was also found in *C. merolae*, an extremophilic red alga as well, which was located in the vicinity of the CP43 protein (Krupnik et al. 2013). While PsbO is present in the PSII-Q4 complex, both of these results reinforce the idea of the binding promiscuity of the PsbQ protein in PSII and its ability to substitute for other lumenal extrinsic proteins. The ability of the PSII-Q4 complex to evolve oxygen, but at a lower rate (66 %) than fully assembled PSII, can be understood in this context.

Previous work has shown that PsbO is the first of the lumenal extrinsic PSII proteins to bind to PSII (Liu et al. 2011; Nowaczyk et al. 2012), and this is consistent with its presence in the PSII-Q4 complex, despite the absence of PsbU and PsbV. The observation that both Q-His PSII complexes were found almost exclusively in the dimeric state suggests that PsbQ binds after PsbO, and that PsbQ binding may facilitate dimerization. This is consistent with cross-linking results that show that PsbQ helps stabilize the
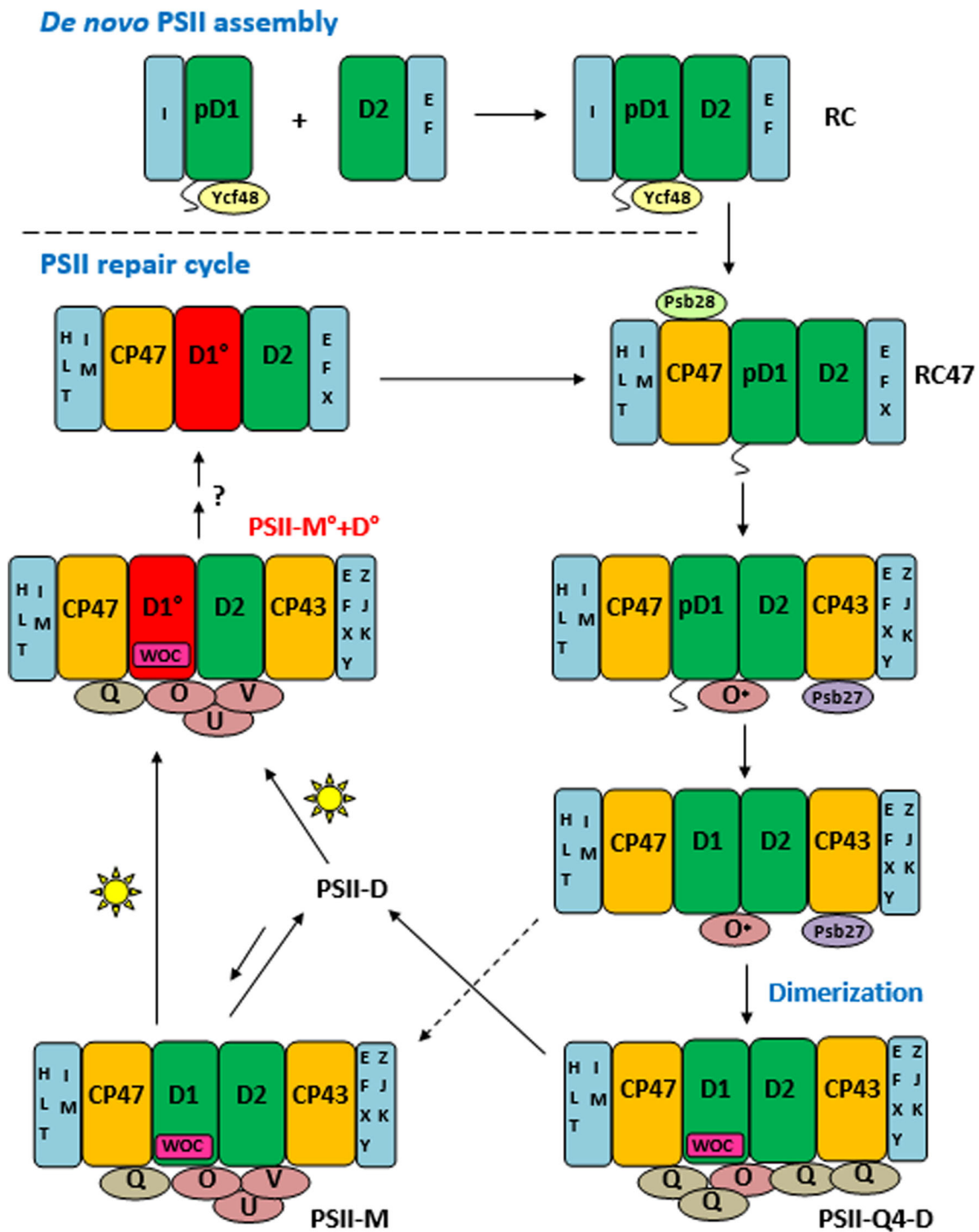
**Fig. 5** A schematic model for PSII assembly and repair. During de novo assembly, the pD1-PsbI-Ycf48 subcomplex joins with the D2-PsbE-PsbF subcomplex to form the reaction center (RC) subcomplex. The RC47 complex is formed after the attachment of CP47 and several low-molecular-weight proteins to RC. Next, CP43 and several more low-molecular-weight proteins bind to the complex. Psb27 associates at this stage, and PsbO is also bound sub-stoichiometrically (indicated by a *asterisk*). After pD1 processing, Psb27 dissociates, PsbO binds stoichiometrically, the WOC is assembled, and dimerization occurs. PSII-Q4 forms at this stage, followed by PSII-D. PSII-D can interconvert with PSII-M. In a parallel pathway, PSII-M may also form directly after Psb27 dissociation, bypassing formation of PSII-Q4. After light-induced D1 damage of PSII-M and PSII-D (represented in red by "D1°"), PSII is partially disassembled. The damaged D1 protein is removed and degraded, a newly synthesized copy is inserted, and re-assembly of active PSII occurs. *M* monomer; *D* dimer. The low-molecular-weight proteins and extrinsic protein names have been shortened from, e.g. "PsbH" to "H," "PsbO" to "O," etc. *Asterisk* indicates sub-stoichiometric binding. The relative positions of different proteins in this diagram are based roughly on the PSII crystal structures and cross-linking results (Suga et al. 2015; Umena et al. 2011; Liu et al. 2011, 2013, 2014)

PSII dimer interface (Liu et al. 2014). PSII-Q4 thus appears to be a late PSII assembly intermediate that is formed just before the binding of PsbU and PsbV. Although we cannot exclude the possibility that PSII-Q4 may also form during the disassembly of PSII (after the dissociation of PsbU and PsbV), the relatively high oxygen evolution rate of PSII-Q4 (66 % of fully assembled PSII) suggests that the majority of PSII-Q4 complexes contain an undamaged D1 protein.

We have incorporated our results into an updated model of PSII assembly (Fig. 5). We suggest that PSII dimerization occurs after dissociation of Psb27 and formation of the WOC. Four copies of PsbQ bind during or immediately after this step, stabilizing this active dimer. Though we did nit observe a monomeric PSII-Q4 intermediate, it is possible that such a complex forms transiently in between Psb27 dissociation and PSII dimerization. The PSII-Q4 dimer is the first intermediate during PSII assembly that is capable of oxygen evolution, albeit at around two-thirds the rate of mature PSII. An active WOC increases PSII vulnerability to oxidative damage. As discussed above, due to the presence of a lipid anchor of PsbQ, multiple copies of PsbQ in PSII-Q4 serve as readily available substitutes for PsbU and PsbV, helping to stabilize the WOC and protect PSII as soon as it gains oxygen-evolving capability. The additional copies of PsbQ must bind relatively weakly, since PsbU and PsbV replace them on the lumenal surface of PSII once they are able to diffuse to their binding sites, forming the fully assembled, fully protected, PSII dimer (PSII-D).Though we hypothesize that formation of PSII-Q4 stabilizes the active dimer until PsbU and PsbV bind, PSII-M and PSII-D are still able to form in the absence of PsbQ (Liu et al. 2014). It is therefore possible that a portion of the complexes bypass PSII-Q4 formation during assembly, as indicated by a dashed line in Fig. 5. Light-induced D1 damage triggers partial disassembly of PSII-M and PSII-D, possibly to the RC47 stage (Nickelsen and Rengstl 2013) (see Fig. 5).It is possible that the PSII-Q4 complex also forms after the dissociation of PsbU and PsbV during PSII disassembly. A new copy of D1 replaces the damaged copy and re-assembly of active PSII occurs.

Changing environmental conditions can alter the rate of PSII damage, making it difficult for the cell to maintain its required rate of real-time energy production. The PSII-Q4 dimer may assist by serving as a pool of nearly assembled PSII that can be rapidly converted to PSII-Din response to shifts in the equilibrium concentration of PSII-D and PSII-M.

In summary, we have isolated a PSII subcomplex (PSII-Q4) with four copies of the PsbQ protein. Based on our results, we conclude that this complex is a late PSII assembly intermediate, formed after the binding of PsbO and before the binding of PsbU and PsbV. This complex helps to stabilize PSII immediately after it becomes capable of water oxidation. Our results provide further evidence for the binding promiscuity of PsbQ and its ability to substitute for other lumenal extrinsic PSII proteins.

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