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Lifetimes of photosystem I and II proteins in the cyanobacterium *Synechocystis* sp. PCC 6803

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ABSTRACT

The half-life times of photosystem I and II proteins were determined using ^{15}N-labeling and mass spectrometry. The half-life times (30–75 h for photosystem I components and <1–11 h for the large photosystem II proteins) were similar when proteins were isolated from monomeric vs. oligomeric complexes on Blue-Native gels, suggesting that the two forms of both photosystems can interchange on a timescale of <1 h or that only one form of each photosystem exists in thylakoids in vivo. The half-life times of proteins associated with either photosystem generally were unaffected by the absence of Small Cab-like proteins.

1. Introduction

Oxygenic photosynthesis in cyanobacteria, algae, and plants is catalyzed mainly by two multi-subunit complexes, photosystem I (PSI) and photosystem II (PSII) that are embedded in the thylakoid membrane. In cyanobacteria, the PSII complex consists of 20 protein subunits, together binding 35 chlorophylls; moreover, extrinsic proteins are located on the lumenal side [1]. According to Blue-Native (BN) gel results and PSII crystal structures, cyanobacterial PSII complexes are present in dimeric and monomeric forms, at least after isolation [2,3], with dimeric forms thought to represent stable, functional complexes, and monomeric complexes possibly representing newly synthesized or damaged/repaired photosystems [4,5]. Monomeric PSI complexes have fewer protein subunits (12) but more chlorophylls (96) than PSII [6]. Cyanobacterial PSI complexes have been found in trimeric and monomeric forms in vitro [2,6–8] and a particularly long-wavelength fluorescence emission form of PSI is found in some cyanobacteria, possibly representing a trimer [8–10], but trimeric PSI has never been observed in the crystal structure or BN protein gels in plants [11,12]. PSI supercomplexes, which consist of trimeric PSI associated with IsiA proteins [13], have also been isolated from cyanobacteria.

An interesting difference between the two photosystems is their stability. The major challenge that PSII complexes face is photodamage caused by oxidizing species. This leads to a rapid turnover of components of PSII complexes: the D1 protein that binds several cofactors including part of P680 turns over on the timescale of about 1 h [14], and other polypeptides close to the PSII reaction center turn over within 15 h [15]. PSI is thought to be much more stable because PSI-catalyzed reactions do not occur at extremely oxidizing redox potentials [16]. However, an accurate turnover rate of PSI proteins has not yet been reported in the literature.

In order to gain comparative knowledge regarding the lifetimes of PSI and PSII proteins, stable-isotope (^{15}N) labeling, BN/SDS–PAGE and mass spectrometry were applied to monitor the fate of old and newly synthesized proteins over time. In addition, Small Cab-like Proteins (SCPs), single transmembrane helix proteins with similarity to part of chlorophyll *a*/*b*-binding light harvesting proteins in plants, have been shown to be involved in PSII chlorophyll recycling [15,17]. There is also evidence that SCPs stabilize PSI complexes [18]. In this study, the lifetimes of PSII and PSI proteins were determined with and without SCPs, and the dynamics of PSII and PSI complexes in their different forms are discussed.

2. Materials and methods

2.1. Strains and growth conditions

The wild-type and Δ*scpABCDE* (SCP-less) strains [19,20] of *Synechocystis* sp. PCC 6803 were grown photoautotropically in liquid

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BG-11 medium [21] at 30 °C in cool-white fluorescent light at an intensity of 75 μmol photons m^{-2} s^{-1}. Cell growth was monitored by measuring the optical density at 730 nm in a 1-cm cuvette using a Shimadzu UV-160 spectrophotometer.

2.2. Isotope labeling and membrane preparation

Cell cultures were grown to an $OD_{730} \sim 0.65$ and were diluted four-fold in BG-11 medium containing 4.5 mM $Na^{15}NO_3$ and 2 mM $^{15}NH_4Cl$. Cells were allowed to continue to grow photoautotrophically under the same conditions as listed above (30 °C, 75 μmol photons m^{-2} s^{-1}), and cell samples were collected at 3, 9, 24 and 48 h after dilution and pelleted by centrifugation. Cell pellets were resuspended in a mixture of 50 mM 2-(*N*-morpholino) ethanesulfonic acid (MES)–NaOH (pH 6.0), 10 mM $MgCl_2$, and 25% glycerol, and broken by Bead Beater (BioSpec Products, Bartlesville, OK). Cell membranes were prepared as described [22] and were stored at −80 °C.

2.3. PAGE

Wild-type and SCP-less membrane samples corresponding to 10 μg of chlorophyll were solubilized in 1% β-dodecyl maltoside for 45 min on ice in darkness; subsequently 0.1 volume of loading solution containing 750 mM aminocaproic acid and 5% Coomassie Brilliant Blue (CBB) G-250, pH 7.5, were added. Protein complexes in the membrane were separated in the first dimension by BN electrophoresis at 4 °C in a 5–14% polyacrylamide gel according to [23]. For the second dimension, the BN gel lanes were incubated for 45 min at room temperature in a solution containing 25 mM Tris–HCl (pH 7.5), 2% SDS, and 10% β-mercaptoethanol. The lanes were then layered onto a 1.5-mm-thick SDS/12–20% polyacrylamide gradient gel containing 7 M urea [24]. The gel was stained with 0.15% CBB R-250 in a solution of 50% methanol, 10% acetic acid, and 40% water. In-gel digestion to produce peptides for analysis by mass spectrometry (LC–MS/MS) was carried out essentially as described [25] using sequencing-grade modified trypsin (Promega/SDS Bioscience).

2.4. Protein analysis

Peptides in trypsin digests were separated using a Dionex Ultimate 3000 liquid chromatography system equipped with both a HPG 3400 M high pressure gradient pump and a LPG 3400 MB low pressure gradient pump together with a WPS3000TB autosampler and a FLM 3100B column compartment. A Bruker MicrOTOF-Q mass spectrometer equipped with an online nanospray source was used for protein identification. Instrumental setups for HPLC and mass spectrometer and data analysis were described earlier [15].

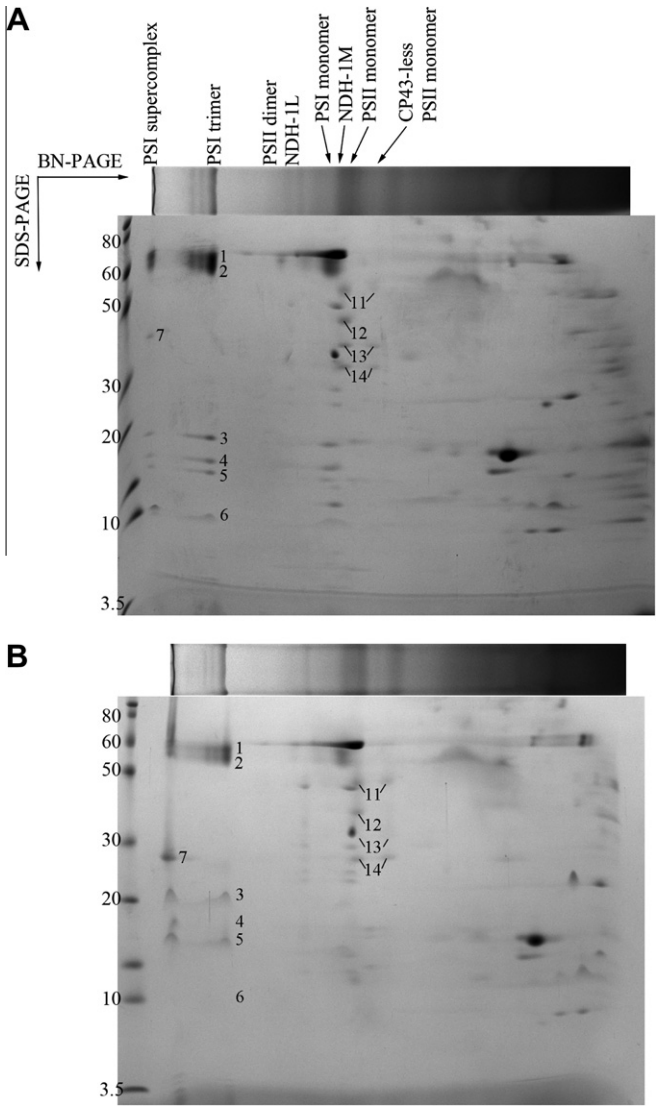

Fig. 1. BN-PAGE followed by SDS–PAGE using membrane proteins from the wild-type (A) and SCP-less (B) strains. The bands on BN-PAGE were visualized by the native chlorophyll and Coomassie Brilliant Blue. SDS–PAGE was stained with Coomassie Brilliant Blue. According to identification of tryptic fragments by LC–MS/MS, protein spots 1–6 were due to the PSI proteins PsaA, PsaB, PsaD, PsaF, PsaL, and PsaE, respectively, spot 7 to IsiA, and spots 11–14 were due to the PSII proteins PsbB, PsbC, PsbD, and PsbA, respectively. Marker proteins are to the left; the indicated size of the components of the protein ladder is in kDa.

3. Results

3.1. Identification of photosynthetic protein complexes and photosynthetic proteins

Membrane protein complexes from wild-type and SCP-less *Synechocystis* sp. PCC 6803 cells were separated by BN-PAGE and then proteins from each individual protein complex were separated by SDS–PAGE (Fig. 1). Various PSI complexes (PSI supercomplex, and trimeric and monomeric PSI complexes) and PSII complexes (complete dimeric and monomeric PSII complexes, and the CP43-less PSII monomer (RC47)) were identified in membranes from both the wild-type and SCP-less strains. Protein complexes other than photosynthetic complexes, such as NADH dehydrogenase (NDH) complexes, were seen as well. The profile of membrane proteins and complexes was very similar to that shown in [2]. In order to determine the lifetimes of PSII and PSI proteins, 2D BN/SDS–PAGE was performed with membrane protein samples from wild-type and SCP-less cells grown in the presence of $^{15}NO_3^-$ and $^{15}NH_4^+$ for a specific time period (0, 3, 9, 24, and 48 h). On the basis of the mass of trypsin fragments, spots 1–6 (Fig. 1) were identified as PsaA, PsaB, PsaD, PsaF, PsaL, and PsaE, respectively, in trimeric PSI, and spots 11–14 (Fig. 1) were found to be PsbB, PsbC, PsbD, and PsbA, respectively, in monomeric PSII. IsiA (protein spot 7 in Fig. 1) was also identified as a component of the PSI supercomplex.

3.2. Dynamics of photosystem I and photosystem II

Labeling and disappearance of unlabeled PSI and PSII proteins were followed over time upon labeling with $Na^{15}NO_3$ and $^{15}NH_4Cl$ (Fig. 2). The cell number increased during the ^{15}N-labeling period, and the total amount of photosystems increased as well. In Fig. 2 we present the percentage of unlabeled protein remaining as a

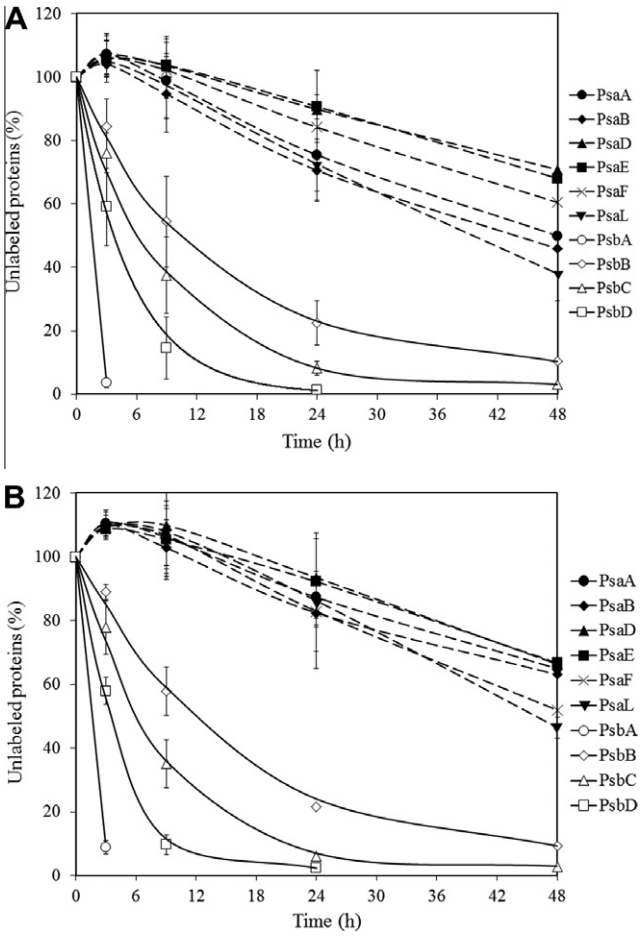

Fig. 2. Turnover of photosynthetic proteins from the wild-type (A) and SCP-less (B) cells. The amount of unlabeled proteins in the wild-type strain was followed during a 48-h period after the start of ^{15}N-labeling. 100% indicates the amount present at the start of labeling. Dashed lines and solid symbols: PSI proteins; solid lines and open symbols: PSII proteins. PsaA: ●; PsaB: ♦; PsaD: ▲; PsaE: ■; PsaF: ×; PsaL: ▼; PsbA: ○; PsbB: ◇; PsbC: △; and PsbD: □. Numbers on the *y*-axis represent the percentage of unlabeled proteins relative to time 0. Results of two independent experiments have been averaged. Error bars represent standard deviations.

function of time, corrected for growth of the culture (OD_{730}) during the time of labeling. In this way, we can compare all data to those at time 0. Interestingly, the amount of unlabeled material initially increased during the first three hours of labeling for all PSI proteins monitored here (Fig. 2A, dashed lines) in the wild-type strain. Under these conditions, PSII proteins degrade rather quickly and a lag in the exponential decrease of unlabeled protein, if present, is difficult to monitor (Fig. 2A, solid lines).

The half-life times of PSI components (Table 1) were determined by monitoring the disappearance of old (unlabeled) peptides in the time period between 9 and 48 h after the start of labeling, whereas for PSII proteins PsbA and PsbD half-life times were calculated from time 0 and for PsbB and PsbC starting at 3 h after labeling. In wild type, the half-life times of PSI reaction center proteins (PsaA and PsaB) were 40 h whereas the other intrinsic PSI proteins, PsaF, which is involved in docking of plastocyanin, and PsaL, which plays a role in formation of the PSI trimer in cyanobacteria [26–28], have half-life times of 50 and 30 h, respectively. The extrinsic proteins (PsaD and PsaE) have longer half-life times (70–75 h) presumably because they can be readily dissociated from damaged PSI and re-used for repaired or new PSI. The fact that extrinsic proteins have longer half-life times than the intrinsic proteins also has been seen for PSII [15].

Table 1
Comparison of the half-life times of PSI and PSII proteins in wild-type and SCP-less strains. The half-life times were calculated from the decrease in the percentage of unlabeled protein correcting for the increase in unlabeled protein shortly after the start of labeling that occurred for the longer-lived polypeptides. Listed are the average results of two independent experiments ± error.

	Half-life time (h)	
Strains	Wild-type	SCP-less
PsaA	40 ± 7	50 ± 4
PsaB	40 ± 7	50 ± 4
PsaD	75 ± 7	50 ± 7
PsaE	70 ± 7	53 ± 4
PsaF	50 ± 7	40 ± 2
PsaL	30 ± 1	30 ± 6
PsbA (D1)	<1	<1
PsbB (CP47)	11 ± 2.5	10.5 ± 1
PsbC (CP43)	6.5 ± 1.5	6 ± 0.5
PsbD (D2)	3.3 ± 1	3 ± 0.3

Perhaps not surprisingly, PSII proteins have much shorter half-life times than PSI proteins. The unlabeled D1 protein had largely disappeared at the 3-h timepoint, and the half-life time of the protein is less than an hour under these conditions (30 °C, 75 μmol photons m^{-2} s^{-1}). The D2 protein was more stable and had a half-life time of a little over 3 h. The CP43 and CP47 proteins, the chlorophyll-binding antenna proteins of PSII, had half-life times of 6.5 and 11 h, respectively. These results are in line with the concept that PSII complexes turn over much faster than PSI complexes. However, compared with the lifetimes of PSII proteins in an earlier study where a much lower light intensity and a light-sensitive PSI-less strain were used [15], the half-life times of PSII proteins were shorter in the experiments reported here, presumably due to the higher light intensity used during the labeling.

As indicated in Fig. 2A, in the wild-type strain there was a 3-h period after the start of labeling during which the amount of unlabeled PSI proteins continued to increase. This cannot be due to a slow incorporation of label into amino acids as D1 polypeptides were almost fully labeled after a 3-h period. Our interpretation of the increase in unlabeled complexes is that there are nascent PSI proteins in thylakoid membranes that are not incorporated into mature PSI complexes. Such proteins will not comigrate with mature complexes on BN-PAGE and therefore will not be counted. Also, the increase in PSI that occurs after correction for growth suggests that the amount of mature PSI per cell increases slightly after the start of labeling, presumably due to the dilution of the culture.

Cyanobacterial PSI is thought to exist in both trimeric and monomeric forms [7,8], and PSII is thought to exist in dimeric and monomeric forms [4,5], based on crystal structures and separation of isolated complexes under non-denaturing conditions. Assuming that this heterogeneity reflects also in vivo conditions, we were interested in following the conversion dynamics of PSI and PSII between multimeric and monomeric forms. For this purpose, the percentage of labeled and unlabeled PSI and PSII proteins from different forms was monitored at different times of labeling. Table 2 shows the percentage of unlabeled proteins at different times of labeling. Each PSI and PSII protein had a similar half-life time when the corresponding complex was in monomeric vs. in multimeric form. Although the extent of labeling for PsaB, PsaE and PsaL proteins was not determined for monomeric PSI at all time points due to the weakness of the bands on the 2-D gel, all of the data obtained were consistent with the observation that there is no difference in the extent of labeling of these proteins in monomeric and trimeric complexes. These results demonstrate that the different forms of PSI or PSII, if relevant under in vivo conditions, have the same half-life times or, more likely, that they are in equilibrium, with the dynamics of PSI and PSII switching between the different forms occurring on a timescale of about an hour or less.

Table 2
Comparison of the percentage of unlabeled PSI proteins in trimeric and monomeric forms and PSII proteins in dimeric and monomeric forms as separated on BN/denaturing gels after 3, 9, 24, and 48 h of labeling. Listed are the average results of one to ten tryptic peptides derived from the proteins below.

		3 h	9 h	24 h	48 h
PsaA	Trimer	96 ± 1	78 ± 2	46 ± 3	14 ± 1.5
	Monomer	95 ± 1	78 ± 1	46 ± 2	13 ± 0.5
PsaB	Trimer	91 ± 1	75 ± 2	43 ± 2	13 ± 1.5
	Monomer	91 ± 2	74 ± 2	43 ± 1	
PsaE	Trimer	94 ± 1	80 ± 1	55 ± 1	18.5 ± 0.1
	Monomer	92 ± 1	78 ± 1	57 ± 1	
PsaF	Trimer	94 ± 0	79 ± 3	51 ± 2	16.5 ± 1.5
	Monomer	93 ± 2	78 ± 3	54 ± 2	11.5
PsaL	Trimer	94 ± 1	76 ± 2	45 ± 1	10 ± 0.5
	Monomer	93 ± 1	78 ± 1		
PsbA	Dimer	2.5			
	Monomer	4.0 ± 1.5			
PsbB	Dimer	80 ± 2	50 ± 3	16 ± 2	3.0 ± 1.5
	Monomer	77 ± 1	47 ± 1	15 ± 2	3.5 ± 1.0
PsbC	Dimer	72 ± 2	37 ± 1	5.5 ± 0.5	1.1 ± 0.2
	Monomer	69 ± 2	34 ± 1	5.5 ± 0.5	1.1 ± 0.1
PsbD	Dimer	59	16 ± 2	0.9 ± 0.1	
	Monomer	58 ± 2	16 ± 1	0.8 ± 0.1	

3.3. Role of SCPs in the photosystems

In a previous study [15], deletion of SCPs in a PSI-less background strain did not change the half-life times of the intrinsic PSII proteins. This is consistent with current results showing that deletion of SCPs in the wild-type background strain did not have a significant effect on the half-life times of the PSII proteins (Table 1, Fig. 2B solid lines). The half-life time of the intrinsic PSI proteins was not much affected by removal of the SCPs either, while the half-life time of the extrinsic PSI proteins (PsaD and PsaE) decreased somewhat (Table 1). Also, the amount of nascent PSI proteins accumulating in the membrane was not significantly changed upon the removal of SCPs (Fig. 2). These results indicate that SCPs do not significantly influence the lifetime of PSI proteins or the presence of putative biosynthetic PSI intermediates.

4. Discussion

Pulse-chase methods using radioisotopes have been widely used to estimate the half-life times of polypeptides that are separated by gel electrophoresis. However, this method is difficult for relatively stable proteins as other proteins with similar electrophoretic mobility are labeled much faster and more intensely. PSI proteins were known to be long-lived [16], but their lifetimes have not been specifically determined. Using stable-isotope labeling combined with mass spectrometry, we can now determine the half-life times of PSI (long-lived) and PSII (short-lived) proteins in parallel.

4.1. Turnover of PSII and PSI proteins

Photosystem complexes and proteins were separated by the BN/SDS–PAGE and identified by LC–MS/MS (Fig. 1). For PSII the half-life times of the reaction center proteins D1 and D2 and the chlorophyll-binding proteins CP47 and CP43 were determined (Table 1). For D1, D2 and CP43, the three polypeptides most directly associated with the PSII reaction center and water splitting, these half-life times were about half of those determined in the PSI-less background strain grown at 4 μmol photons m^{-2} s^{-1} [15]; the difference in the CP47 lifetimes in the two conditions was less pronounced (30% difference). The difference in light intensity between the two studies was almost 20-fold. The reason why the half-life time of PSII components does not inversely correlate with the light intensity most likely is that the redox state of PSII components and the plastoquinone pool, which is rather oxidized in the wild type in darkness or at low light intensity but becomes readily reduced in the PSI-less strain at low light intensity [29], does not become reduced in wild type until at higher light intensity because an abundance of PSI is present in the same membrane. A more reduced plastoquinone pool is correlated with more photoinhibition [30].

As indicated in Table 2, throughout the labeling period the PSI and PSII proteins have the same percentage of unlabeled peptides when their corresponding photosystems are in monomeric vs. multimeric form. The time resolution of this experiment is on the order of an hour, which is similar to the lifetime of the D1 protein but much shorter than the lifetime of PSI proteins. These results suggest that either the PSI and PSII complexes dynamically interchange between monomers and multimers or that multimeric arrangements found in isolated complexes and detected on BN gels or in crystals are artifactual. Another possibility is that the lifetimes of proteins in monomeric and multimeric complexes are identical, but this would suggest that there may not be a functional difference between the various types of complexes.

Whereas the evidence for trimeric arrangements of PSI in vivo is scarce, there is evidence for a functionally relevant distinction between PSII monomers and dimers from freeze-fracture analysis of plant thylakoids: PSII dimers are in grana regions and PSII monomers are in unstacked thylakoid regions [31,32]. As PSII in unstacked regions of the plant thylakoid may correspond to photosystems that are undergoing repair [33,34], the interchange between monomeric and dimeric PSII complexes in the membrane indeed may be rapid. However, if there are nascent PSII components that are stable in the membrane for several hours, then the interchange between monomers and dimers might not be that rapid for all PSII proteins. Therefore, it is unclear at the present time whether exchange between monomeric and dimeric PSII is rapid under in vivo conditions, or whether only one of these forms is prevalent under physiological in vivo conditions.

For PSI a functionally relevant distinction between the monomeric and trimeric forms of the photosystem has not been made, and in view of the highly stable PSI proteins and the identical half-life times of PSI proteins in PSI monomers and trimers, it is very well possible that trimeric PSI complexes in *Synechocystis* are an isolation artifact.

4.2. Chlorophyll in the photosystems

In *Synechocystis*, PSI is an abundant membrane protein complex in the thylakoid membrane binding ~80% of the chlorophyll in cells [35]. ^{15}N-labeling experiments demonstrated that the lifetime of chlorophyll in the wild-type cells was very long (>200 h), whereas it was much shorter (80 h) in PSI-less cells [17]. This suggests that PSI-associated chlorophyll has a long lifetime. If one assumes a lifetime of PSI-associated chlorophyll of >200 h, this lifetime is more than five times that of the PSI chlorophyll-binding proteins (PsaA and PsaB). This suggests that chlorophyll is recycled upon degradation of PSI chlorophyll-binding proteins and can be reincorporated into new or existing complexes. The recycling of chlorophyll is also seen in PSII [15,17].

4.3. SCPs, chlorophyll, and photosynthetic proteins

As shown in Table 1, the lifetimes of PSI and PSII chlorophyll-binding proteins were not affected by the removal of SCPs. Moreover, monomers, trimers and supercomplexes of PSI were observed upon BN-PAGE regardless of the presence of SCPs. However, in an earlier report ScpC (HliA) and ScpD (HliB) proteins were found to be associated with trimeric PSI complexes, and SCPs stabilized PSI trimers at high light intensity [18]. However, the identified PSI trimers associated with HliA and HliB in [18] were not merely PSI

trimers but PSI supercomplexes that contain IsiA proteins. Interestingly, ScpB (HliC) was reported to be associated with PsaL [18], which is involved in formation of PSI trimers; however, the half-life time of the PsaL protein was unchanged upon deletion of SCPs in this study. The discrepancies between the two studies could be caused by different growth conditions as SCPs and IsiA are stress-inducible proteins. However, in our study there is no clear evidence that SCPs stabilize PSI complexes.

In conclusion, the half-life times of PSI proteins are on the order of a couple of days and are thereby much longer than those of PSII proteins. The results presented here illustrate the dynamic nature of photosynthetic complexes in the thylakoid membrane, with apparently rather rapid exchange between monomers and dimers in PSII, if both exist in vivo. If PSI trimers exist in vivo, then they are in rapid equilibrium with monomers as the two forms have the same half-life times. These results illustrate the usefulness of stable-isotope labeling and native-gel approaches to monitor the half-life times and putative multimerization kinetics of relatively stable complexes.

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