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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].

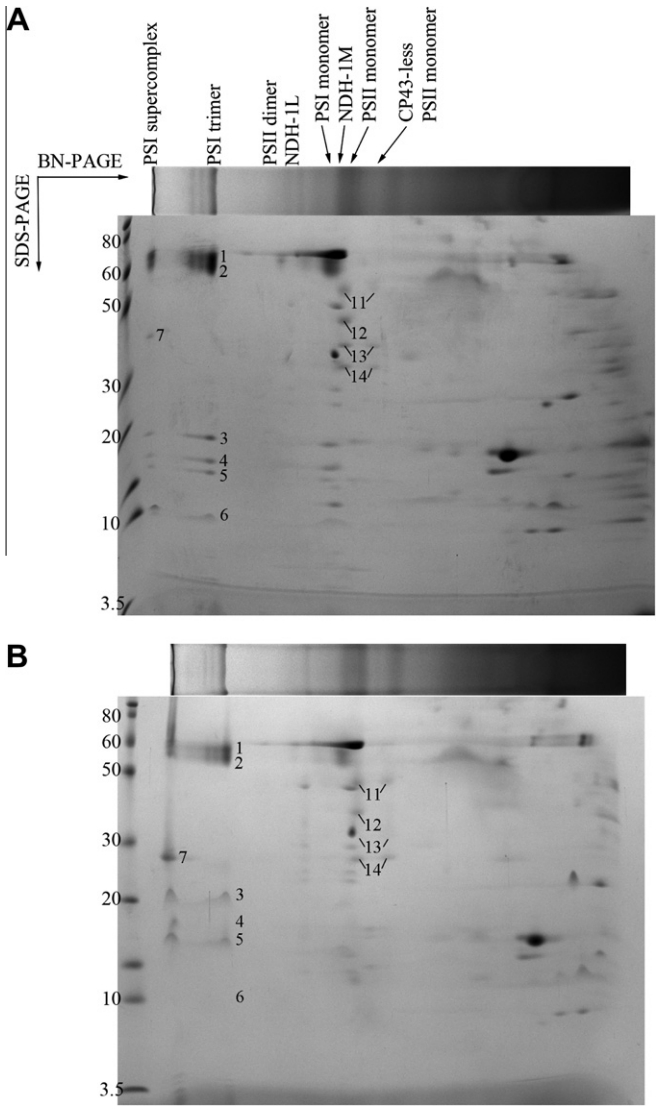

Fig. 1. BN-PAGE followed by SDS–PAGE using membrane proteins from the wild-type (A) and SCP-less (B) strains. The bands on BN-PAGE were visualized by the native chlorophyll and Coomassie Brilliant Blue. SDS–PAGE was stained with Coomassie Brilliant Blue. According to identification of tryptic fragments 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