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Stabilization of Trimeric PSI within *Thermosynechococcus elongatus* via Chlorophyll *a* and Carotenoids Located in Monomer-Monomer Interface Area

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MONOMERIC PSI is found within thermophilic cyanobacteria, i.e., *Thermosynechococcus elongatus* either in case of iron deficiency or deletion of PsaL subunit. Previous investigations pointed to the role of PsaL in oligomerization of monomeric complexes. Successful isolation of monomeric PSI was achieved only via salt treatment of thylakoid membrane at high temperature (50°C).

Here, monomeric PSI was obtained through splitting the purified trimeric PSI *in vitro*. Selective elimination of carotenoids and Chlorophyll *a* located in monomer-monemer interface area was performed using a mixture of hexane with different organic solvents. Hexane-dimethyl sulfoxide mixture was considered the best organic solvent for removal the interface carotenoids and chlorophyll *a*, where it splatted trimeric PSI completely. Also, the role of carotenoids and chlorophyll *a* on stabilization of trimeric PSI was proven by sucrose density gradient and blue native gel. 77K spectroscopical emission fluorescence measurements of investigated PSI showed blue shift peaks due to monemerization as well as formation of uncoupled chlorophylls. The efficiency of organic solvents on removing carotenoids molecules was higher than chlorophyll molecules, where about 12/96 chlorophyll molecules and 12/22 carotenoids molecules have been eliminated in case of complete splitting of trimeric PSI.

Keywords: Trimeric Photosystem I (PSI), *Thermosynechococcus elongatus*, Chlorophyll *a*, Carotenoids.

Introduction

Photosystem I (PSI) is considered the largest recorded mutisubunits protein complex that exists in the thylakoid membrane of plant, algae and cyanobacteria. PSI acts as energy converter that catalyzes the electron transfer from plastocyanin or cytochrome C_6 to ferredoxin (Rögner et al., 1990a). Under normal condition, PSI of *Thermosynechococcus elongatus* is found in trimeric form, each monomer has a molecular mass of 1068kDa (Jordan et al., 2001). Due to large cofactors content, it is estimated as 30% of its total molecular mass of PSI complex. Cyanobacterial PSI contains long-wavelength Chls (LWC) that absorb light energy lower than that of reaction center (P700) (Gobets & Grondelle, 2001; Karapetyan et al., 2006 and Schlodder et al., 2007). El-Mohsnawy et al. (2010) found that monomeric PSI contains less LWC than that found in trimeric PSI of *Thermosynechococcus elongatus*.

Structural analysis by X-ray of trimeric PSI from the thermophilic cyanobacterium *Thermosynechococcus elongatus* revealed existing of 12 protein subunits that carries 127 cofactors (Jordan et al., 2001; Fromme et al., 2001 and Fromme et al., 2003a). Ninty chlorophylls (Chls) and 22 carotenoids (Car) are the main capture pigment in antenna. 6 Chls, two phylloquinones, three pigments iron sulfur (4Fe-4S) clusters are located in reaction center that functions as electrons pumping and transferring to ferredoxin (FD) (Jordan et al., 2001 and Grotjohann & Fromme, 2005). The 77K emission spectral differences between monomeric and trimeric PSI of *Arthrospira platensis* might indicate an energy dynamic equilibrium of the monomer/trimer (Karapetyan et al., 1999; Kruip et al., 1999 and Karapetyan, 2008). Although PSI complex of cyanobacteria shows high similarity to those of higher plants and algae, several variations have been observed (Jordan et al., 2001; Ben-Shem et al., 2003 and Nelson & Yocum, 2006).

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Subunits PsaM and PsaX are detected only in cyanobacteria, while subunits PsaG and PsaH as well as light harvesting complexes are missing. While subunits PsaI and PsaL are responsible for formation and stabilization of the PSI trimer in cyanobacteria (Chitnis, 2001 and Amunts et al., 2007), subunit PsaL was thought to have different structural functions in cyanobacteria and higher plants (Chitnis, 2001 and Ben-Shem et al., 2003). Cyanobacterial PSI was suggested to have preferably a role on stabilization the trimeric complex (Fromme et al., 2003a, b and Fromme, 2003). In contrast, PsaL in higher plants binds to peripheral Lhca1-Lhca4 complexes (Boekema et al., 2001; Ben-Shem et al., 2003 and Klimmek et al., 2005) and/or PsaH which consequently prevents trimerization process in higher plants (Sener et al., 2004 and 2005). In cyanobacteria, most carotenoids molecules are bound to the photosystem complexes (Guskov et al., 2009 and Vajravel et al., 2016) and also to some protein subunits like the high-light inducible proteins (Komenda & Sobotka, 2016). Besides protein binding carotenoids, a substantial fraction of nonprotein-bound xanthophylls are found in membranes (Gruszecki & Strzalka, 2005 and Domonkos et al., 2013). β-carotene is an important pigment within PSI complex through its influencing structure (Toth et al., 2015) and function (Bautista et al., 2005). Moreover, recent results on *Synechocystis sp*. PCC 6803 showed that xanthophyll deficiency (i. e., deficient of oxygenated carotene derivatives) resulted in reduction of the amount of PSI oligomers (Toth et al., 2015).

Physiological responses are always accompanied by PSI oligomerization, where the trimeric PSI complex is more tolerant than monomeric complex against high temperature, where the α-helices in monomers are more susceptible to temperature elevation than the respective α-helices in trimers. For this reason, oligomerization might be an effective strategy in thermophilic cyanobacterium *Thermosynechococcus elongatus* to enhance thermostability of PSI complexes (Shubin et al., 2017). Environmental factors can also influence the oligmerization of PSI complex, where under iron deficiency, trimeric PSI complex is splitting into monomeric complex (Ivanov et al., 2006 and El-Mohsnawy, 2014). Aso, a PsaL-deficient mutant (ΔPsaL) could produce only monomeric PSI and was not able to form PSI trimers (Kłodawska et al., 2015).

Trimeric from of PSI in *Thermosynechococcus elongatus* is the stable form that has been resolved by three-dimensional (3D). In spite of successful extraction of monomeric PSI from, understanding the mechanism of stabilization of the three monomers within trimeric PSI is critical issue for further application especially that concerning of hydrogen production. The present work investigates the role of chlorophyll *a* and carotenoids in the stabilization of trimeric PSI in *Thermosynechococcus elongatus.*

Materials and Methods

Cultivation conditions

Investigated *Thermosynechococcus elongatus* cells were cultivated on BG11 medium in 20L photobioreactor at 50°C (Slavov et al., 2009 and Shubin et al., 2010).

White light was provided in 100μE.$m^{-2}s^{-1}$ and increased gradually cultural optical density to reach 150μE.$m^{-2}s^{-1}$. Harvesting occurred at OD_{750nm} of 2.5-3. After 2 washing cycles, harvested cells were disrupted by pressing cells at 2000 psi using Parr bomb at 4°C (Rögner et al., 1990a, b and Wenk & Kruip, 2000). Thylakoid membrane was suspended in HEPES buffer (20mM HEPES [pH 7.5], 10mM $CaCl_2$, 10mM $MgCl_2$ and 0.5M mannitol) reaching final chlorophyll concentration of 0.75μg/μl followed by homogenization five times and centrifuged at 8100g for 20min. Thylakoid membrane was exposed to 3 washing cycles using HEPES buffer followed by suspending in HEPES buffer to reach chlorophyll concentration of 1μg/μl. Trimeric PSI complex was isolated by stirring thylakoid suspension with 0.6% n-Dodecyl β-D-maltoside (β-DM) at room temperature for 20 min followed by ultracentrifugation at 180000g for 1h. Filtrate containing trimeric PSI complex was pass through two chromatographic steps, hydrophobic interaction chromatography (HIC) and ion exchange chromatography (IEC). After purification, PSI complex was suspended in HEPES buffer (pH 7) containing 0.03% β-DM and concentrated before store.

Removing detergent and lyophilization trimeric PSI

β-DM elimination was performed according to Biggins & Mathis (1988) and Biggins (1990) with some modifications. Concentrated pure PSI complex was washed 4 times by distilled water

(for removing sugar, salts, detergent, buffer etc.) using AMICON centrifuge tube 50K. Samples were concentrated to chlorophyll concentration of 3µg/µl. About 90µg Chl samples were equally distributed into 18 evacuated tubes followed by freezing at 77K using liquid nitrogen. Tubes were transferred into vacuum (LYOVAC GT 2, LeyBold-Heraeus) and freeze-dried overnight at dark condition.

Extraction of surface pigments by several organic solvents

Dried samples were arranged in 6 groups, each group has 3 replicates; according to the following categories:

1. PSI Tri treated with 400µl Hexane
2. PSI Tri treated with 400µl Hexane with 0.05% methanol (MeOH)
3. PSI Tri treated with 400µl Hexane with 0.05% Dimethyl sulfoxide (DMSO)
4. PSI Tri with 400µl Hexane treated with 0.05% acetone
5. PSI Tri treated with 400µl Hexane with 0.05% ethanol (EtOH)
6. PSI Tri reference, without any treatment.

Each sample (1-5) was suspended in 400µl extraction solvent (within 30sec) followed by centrifugation at 13000/min for 2min. After centrifugation, filtrates were collected and PSI complexes were left in air to dry. All protein complexes and untreated PSI Tri (group 6) were suspended in 300µl HEPES buffer (0.1 M HEPES 7.5, 10mM $MgCl_2$, 10mM $CaCl_2$, 0.03% ß-DM, 0.5 M mannitol). For comparison with untreated monomeric PSI complex, lyophilized PSI monomer that purified according to El-Mohsnawy et al. (2010) was used.

Characterization of treated PSI complexes and extracted pigments

Blue native gel electrophoresis

Blue native fractionation by gel electrophoresis was performed according the description of Cline & Mori (2001) with some modifications. PSI samples were mixed with equal volume of CBB-solution (750mM ACA, 5% (w/v) Serva Blue G, 10% (v/v) glycerol), shaked for 20min and loaded onto gel. Electrophoresis was left overnight at 65V and 20mA and 4°C. A mixture of 50mM Tricine, 0.02% (w/v) Coomassie-Blue G250 and 15mM Bis-Tris was used as cathodic buffer, the anodic buffer contained 50mM Bis-Tris·HCl pH 7.0. Gel-page was partially destained by shaking overnight in washing buffer containing 20mM Na-phosphate buffer pH 7.8 and 10mM NaCl.

Sucrose density gradient

Twenty % sucrose in HEPES buffer containing 0.03% ß-DM was frozen and slowly defrozen overnight at 10°C.

Fifty µl of each treated PSI complexes was dropped carefully on the top of the defrozen sucrose gradient. Gradients were exposed to centrifugation at 25000rpm for 18h at 4°C (SW28-Rotor ultracentrifuge, Beckman).

Room temperature absorption

Absorption spectra of PSI complexes were achieved by UV-3000 (Shimadzu, Japan) spectrophotometer. Samples were scanned in room temperature in the range of 350nm to 800nm. Data were not corrected for spectral sensitivity.

77K fluorescence emission using 440nm and 500nm actinic lights

Fluorescence emission spectra were carried out using SLM-AMINCO Bauman, Series 2 Luminescence spectrometer. PSI complexes were diluted to about of 3µg Chl/ml in HEPES buffer containing (0.02% ß-DM and 60% glycerol) (El-Mohsnawy et al., 2010). Samples were immersed in liquid nitrogen and excited by 440nm actinic light (for exciting Chl) and 500nm actinic light (for exciting carotenoids). Fluorescence emission spectra were recorded in the range of 600 to 800nm with 1nm step size and 4nm bandpass filter.

Pigments content estimation

Ten µl of PSI complexes was mixed with 990µl 80% (v/v) acetone. Mixture was centrifuged at 11500g for 2min. The optical density was recorded at (665nm and 649nm). Chl *a* concentration (mg/ml) was estimated according to the equation of Lichtenthaler (1987), while carotenoids were estimated according to equation of Liaaen & Jensen (1971).

Results

Fractionation of splitting photosystem complexes

Since PSI trimer and monomer complexes have different molecular weight, they are easily separated and distinguished *via* sucrose density gradient. As shown in Fig. 1, tubes No. 1, 2, 4

and 5 have two definite bands that gave strong evidence for presence of both PSI complexes (monomer and trimer). Concerning tubes 1, 2 and 5, there are faint upper bands, which give conclusion of low amount of PSI monomers due to solvents treatments of PSI trimers, by hexane, hexane-methanol and hexane ethanol solvents. In tube 4, trimeric PSI treated with hexane-acetone mixture, the upper band (PSI monomer) looked more concentrated. While in tube no 3, treatment trimeric PSI by hexane-DMSO mixture, led to formation of only one concentrated upper band (PSI monomers) and completely disappearing of trimeric PSI.

Blue Native-PAGE is considered a powerful separating tool for native protein complexes based on size without denaturing them. Trimeric PSI complexes treated with different organic solvents were separated by BN-PAGE into two visualized bands in case of lane 1, 2, 4 and 5. These two bands are corresponded to PSI trimers (upper band) and PSI monomers (lower band). Blue native fractionation gel results came almost identical to be obtained by sucrose density gradient, where lanes 1, 2 and 5 (trimeric PSI treated with hexane, hexane-methanol mixture and hexane-ethanol, respectively) showed faint lower bands corresponding to monomeric PSI. Compared to Lane 1, 2 and 5, lane 4 (trimeric PSI treated with hexane-acetone mixture) showed concentrated lower band (PSI monomer). On the other hand, lane 3, trimeric PSI treated with hexane-DMSO, showed completely disappearing of upper band, PSI trimers and highly concentrated lower band (Fig. 2).

Spectroscopical characterization of splitting PSI complexes

Absorption variations at room temperature

As shown in Fig. 3, absorption analysis of PSI trimer complexes treated with different organic solvents showed remarkable reduction of carotenoids absorbance in the range of 470nm to 510nm. Untreated monomeric and trimeric PSI complexes have nearly the same values that give indication for having nearly the same carotenoid contents. All treatments exhibited almost the same behavior at this region. In contrast, trimeric PSI complexes treated with hexane-DMSO mixture showed 25% reduction at red region (680nm) compared to untreated PSI trimer complexes, which indicates reduction of chlorophyll content due to hexane-DMSO treatment. PSI treated with Hexane-acetone also exhibited reduction of chlorophyll absorbance reaching 93% compared to control.

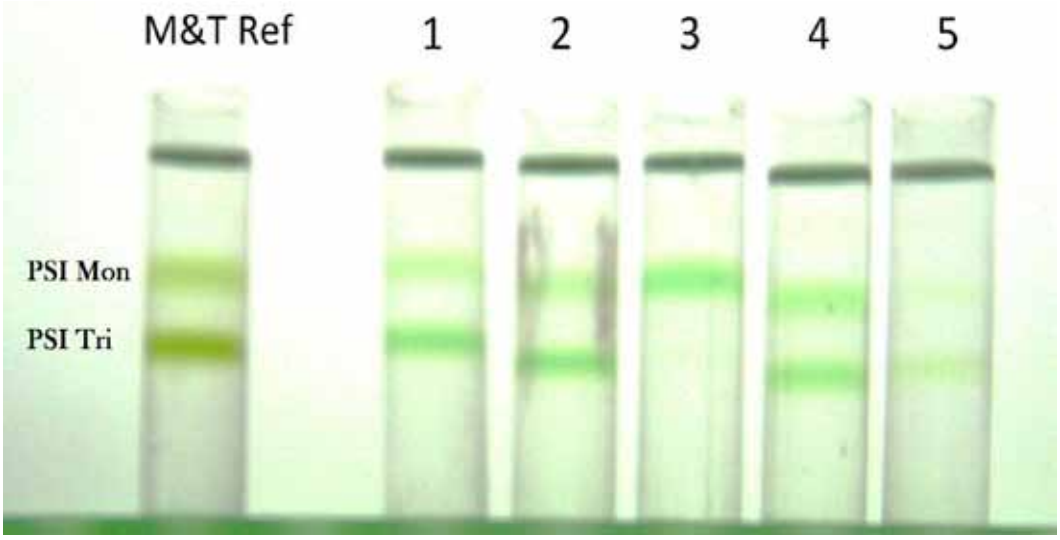

Fig. 1. Sucrose density gradient of PSI trimer complexes treated with different organic solvents; 1: PSI trimer treated with hexane, 2: PSI trimer treated with 0.05% methanol in hexane, 3: PSI trimer treated with 0.05% DMSO in hexane, 4: PSI trimer treated with 0.05% acetone in hexane and 5: PSI trimer treated with 0.05% ethanol in hexane (Both untreated purified monomeric and trimeric PSI were taken as reference in the left side).

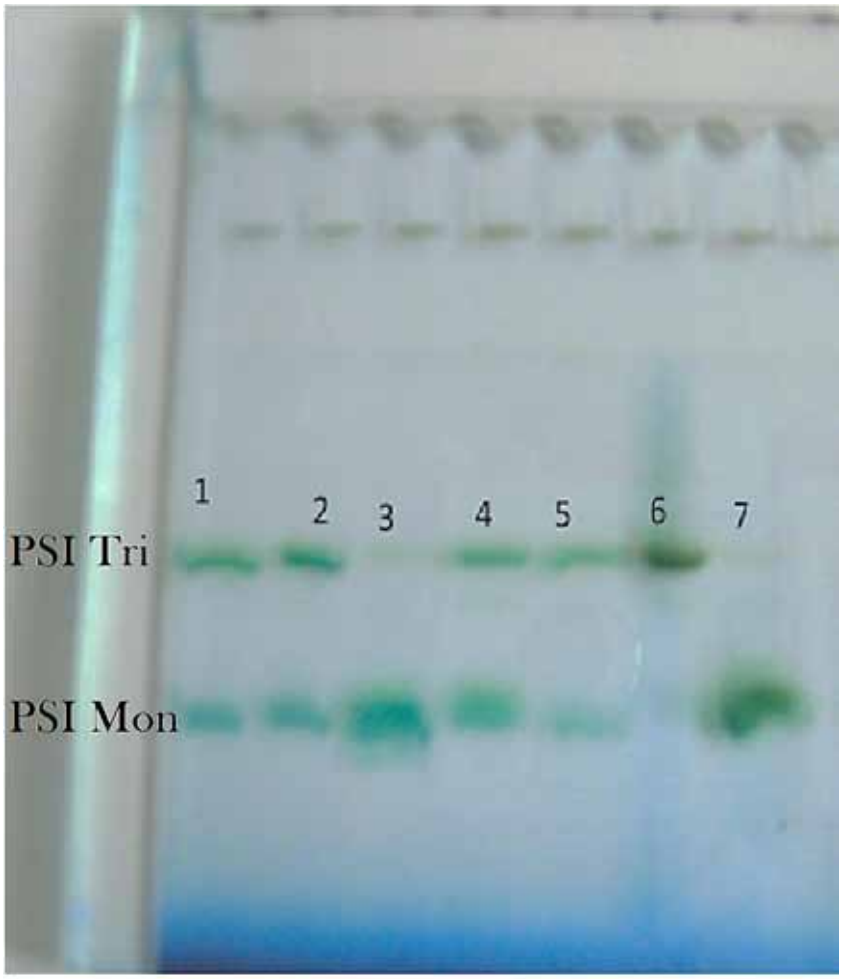

Fig. 2. Blue native gel electrophoresis of PSI trimers treated with different organic solvents; 1: PSI trimers treated with hexane, 2: PSI trimers treated with 0.05% methanol in hexane, 3: PSI trimers treated with 0.05% DMSO in hexane, 4: PSI trimers treated with 0.05 % acetone in hexane, 5: PSI trimers treated with 0.05% ethanol in hexane, 6: Untreated purified trimeric PSI and 7: Untreated monomeric PSI.

Fluorescence emission analysis at 77K

Seventy seven K fluorescence emission spectral analysis is considered a sensitive technique that can record small variations of chlorophyll molecules within trimeric PSI complexes. Untreated trimeric PSI complex showed the maximum emission peak at 733nm that blue shifted to 726nm in case of untreated monomeric PSI complex. By treating trimeric PSI complex with organic solvents, 5nm blue shifted reaching 728nm was observed due to treating by hexane-acetone and hexane-ethanol mixtures. In contrast, only 2nm blue shift was recoded in case of treatment by hexane or hexane-methanol mixture. By treating PSI trimer complex with hexane-DMSO mixture, 8 nm blue shifted was detected reaching 727nm close to that of monomeric PSI. Additionally, a new peak was observed in all cases at 679nm. High intensity was recorded in case of PSI trimer complex treated with hexane-acetone, while the lowest intensity was observed in case of PSI trimer complex treated with hexane-DMSO (Fig. 4A). As shown in Fig. 4B, fluorescence emission spectra of trimeric PSI complex and that treated with hexane-DMSO mixture were recorded at the same wavelength, 725nm, whereas, the intensity was remarkably influenced by organic solvent treatment.

Absorption spectra of extracted pigments

Obtained pigments extracted from trimeric PSI complexes by different organic solvents were examined by spectrophotometer in the range from 300nm to 700nm. Recorded spectra showed high carotenoids absorbance peaks at 446nm and 473nm. Moreover, hexane and hexane

methanol extracts showed the highest absorption compared with other extractions, while the lowest absorptions were recorded for hexane-acetone and hexane-ethanol. Additional two peaks were detected at 426nm and 656nm that reveals presence of chlorophyll in the extract in case of extraction by hexane-DMSO (Fig.5).

Pigments estimation of treated PSI complexes

Variations (%) of chlorophyll and carotenoid contents of treated PSI trimers after using different organic solvents were demonstrated in Fig. 6. Chlorophyll contents are partially affected by solvent treatment, where the missing molecules ranged from 6% in case of hexane and hexane-ethanol to 12% in case of hexane-DMSO. Compared to chlorophyll, carotenoids were highly influenced by organic solvents treatment, where the missing carotenoids ranged from 63.6% in case of hexane-methanol to 52% in case of hexane-ethanol. The obtained results pointed to the efficiency of hexane-DMSO to eliminate more chlorophyll molecules than other solvents (Fig. 6).

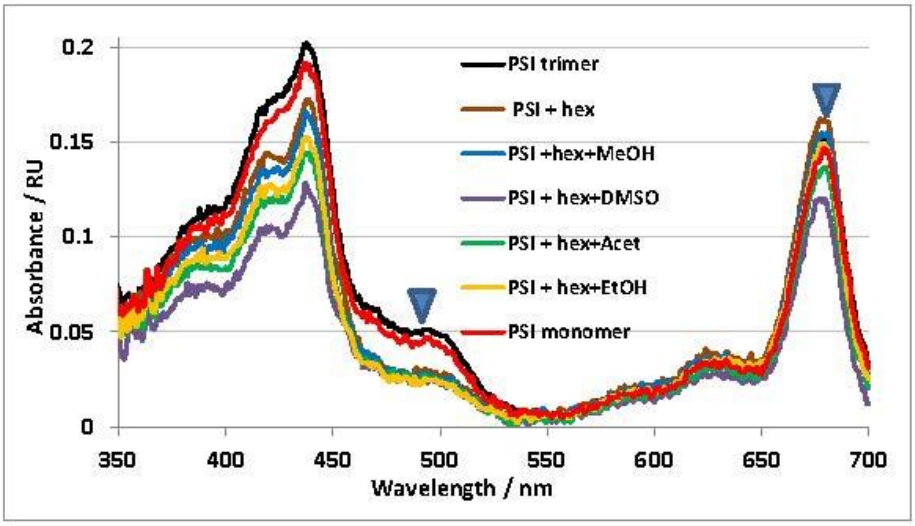

Fig. 3. Room temperature absorption spectral scan (350nm-700nm) of PSI trimer complexes after solvents treatment.

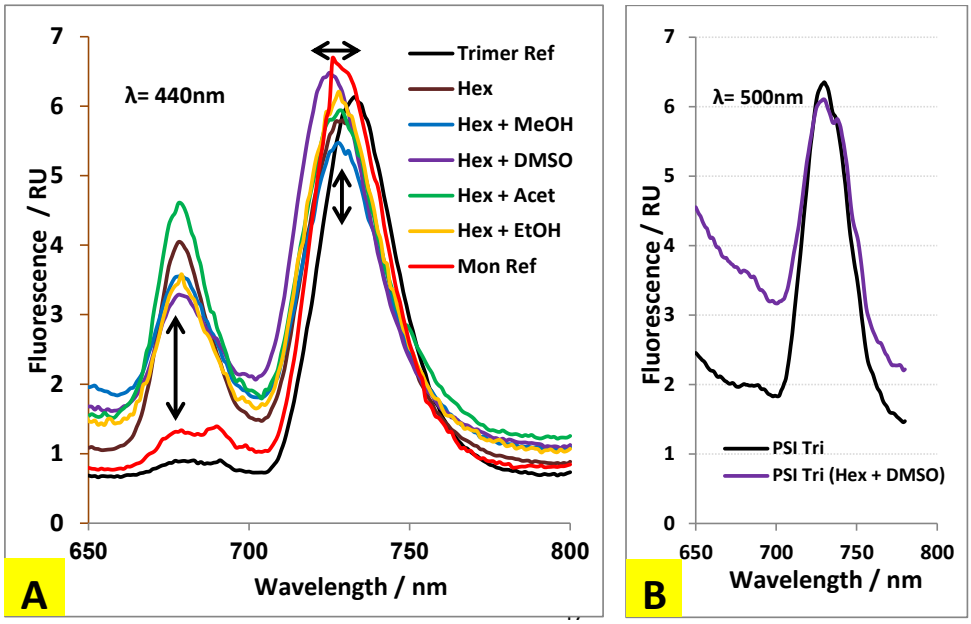

Fig. 4. Emission fluorescence spectral analysis of trimeric PSI complexes after treatment by different organic solvents using 440nm actinic light (A) and Untreated trimeric PSI and trimeric PSI treated by DMSO using 500nm actinic light (B).

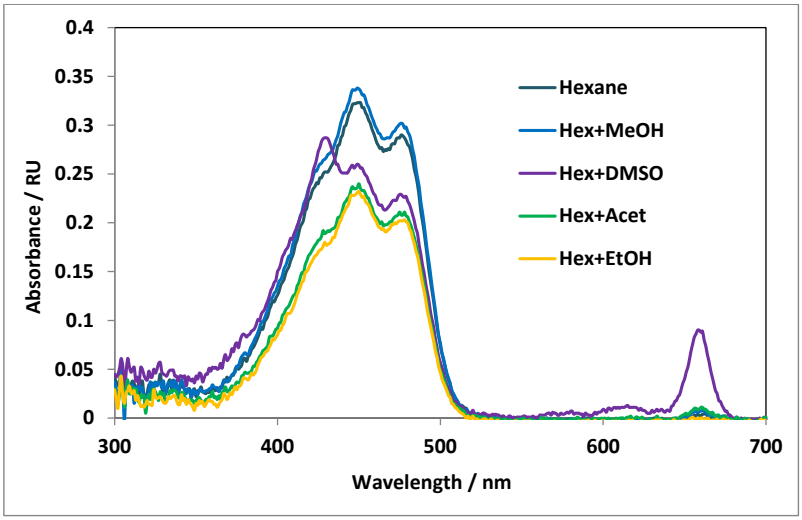

Fig. 5. Absorption spectral scan of extracted pigments by different organic solvents.

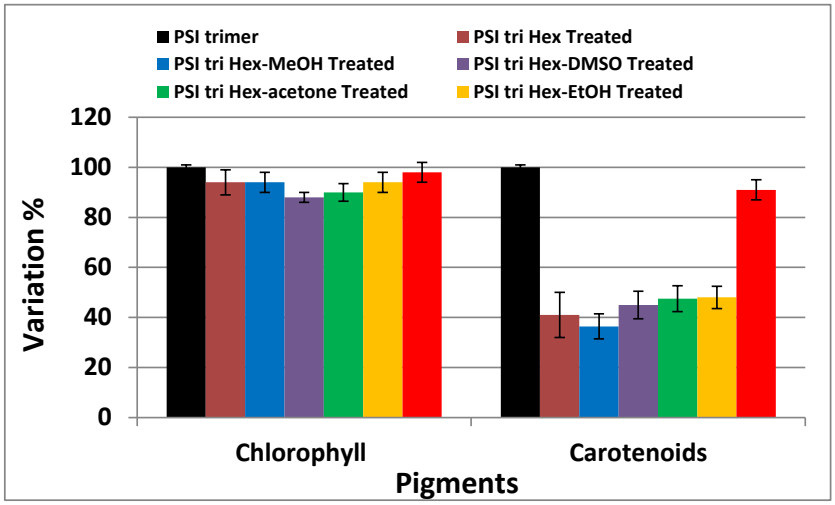

Fig. 6. Pigment content variation (%) of trimeric PSI complexes treated with different organic solvents compared to untreated PSI complex (Monomeric PSI was compared as reference).

Discussion

Studies performed on cyanobacteria reveal that trimeric PSI complex is detected on species that exposed to high light intensity and/or high temperature (Kłodawska et al., 2015). Although PSI trimers that investigated by high resolution X-ray analysis answered several question concerning complex structure and pigments distribution, these investigations cannot detect the stabilization mechanism of PSI trimers in *Thermosynechococcus elongatus* (Jordan et al., 2001).

Trimerization of PSI is thought to be accompanied by a central protein subunit, PsaL protein (Chitnis & Chitnis, 1993 and Klodawska et al., 2015). Not only PsaL is suggested to be the stabilizer subunit required for trimeric formation but PsaI and PsaM as well as three β-carotene molecules bound to PsaL also might play structure importance (Grotjohann & Fromme, 2005 and Toth et al., 2015). Also, Ca^{2+} is assumed to have a stabilization role for trimeric formation (Jordan et al., 2001). Long-wavelength Chls (LWCs) were estimated to be 5-10% of total chlorophyll content (Mazor et al., 2014). El-Mohsnawy et al. (2010) suggested that LWCs should be located at monomer- monomer interface area, where both 5K absorbance and 77K fluorescence showed blue shift peaks in case of PSI monomer in *Thermosynechococcus elongatus*, which are

considered a sensitive sensor for identifying either monomeric or trimeric complexes (El-Mohsnawy et al., 2010). Under physiological stresses like iron deficiency, PSI trimer complex was splitting into monomer and new chlorophyll captures protein known as IsiA was overexpressed to protect the photosynthetic apparatus (Ivanov et al., 2006 and El-Mohsnawy, 2014).

Sucrose density gradient is an efficient tool for separating photosynthetic complexes (Lax et al., 2007), so produced monomeric PSI complexes was easily separated from unsplitted trimeric PSI complexes after organic solvent treatments. Additional evidence for partially and completely splitting of trimeric PSI complexes into monomer complexes was the blue native gel electrophoresis (Kubota et al., 2010).

Combination the obtained absorption spectra data of trimeric PSI complexes after solvents treatments (Fig. 3) with those of extracted pigment absorption (Fig. 5) and chlorophyll contents (Fig. 6), it could be reached to the following conclusion. Due to different polarities of used organic solvents, the extracted amounts of carotenoids and chlorophylls depend upon solvent polarity, time of incubation and solvent/ pigment concentration (Moran & Porath, 1980). Because hexane is considered non-polar solvent that used either singly or as the dominant solvent mixture components, the efficient solubility of carotenoids were very high, so the extraxted carotenoids molecules were very high that ranged from 52% to 63%. Although chlorophyll molecules did not remarkably influenced by treated solvents, chlorophyll molecules exhibited different responses against solvent type. It was obvious that the efficiency of hexane-DMSO on chlorophyll removal was very high, compared to other solvents (Fig. 3-6). It should point that by increasing DMSO concentration in hexane-DMSO mixture, liberated chlorophyll molecules dramatically increased (data not shown).

Fluorescence emission spectra of treated and untreated PSI trimer complexes exhibited blue shift that indicates reduction of LWCs due to dissociation into monomeric PSI. These results came in agreement with that reported by El-Mohsnawy et al. (2010). Additionally the obtained blue shifts varied in response to treated organic solvents, which gives strong evidence for presence of different monomeric and trimeric PSI ratios so it could be concluded that the dissociation degree of trimeric PSI is type of organic solvent dependent. This conclusion comes in agreement with that published by Zakar et al. (2018), who referred the emission fluorescence differences of investigated cyanobacteria to different monomeric and trimeric PSI ratios. Development of new emission peaks at 679nm in response to solvent treatment reveals to presence of uncoupled chlorophylls. These uncoupled chlorophylls lost their position in antenna network but still loosely bound to their protein subunits, so these uncoupled chlorophylls emit fluorescence at 679nm (Albus et al., 2010). It is clear that the efficiency of hexane-DMSO mixture on removal the monomer-monomer interface chlorophylls was higher than that of other solvent mixtures. This conclusion was proven by estimation of chlorophyll concentration of extracts (Fig. 5) and treated PSI trimer complexes (Fig. 6). Evaluation the antenna system before and after hexane-DMSO treatment shown in Fig. 4B revealed existing of some carotenoids molecules within antenna complex and the slight decrease of amplitude around 500nm is probably related to the missing of some carotenoids molecules (Wientjes et al., 2012).

Based on available data, all solvent mixtures can eliminate high carotenoid molecules as shown in Fig. 3, 4B, 5 and 6, while hexane-DMSO is characterized by elimination chlorophyll molecules more than other treatments (Fig. 3, 4A, 5 and 6). By combination these date with that shown in Fig. 1 and 2, it could be concluded that both chlorophyll and carotenoid molecules should be involved on stabilization the trimeric complexes. This hypothesis is supported by data published by Fromme et al. (2001) and Sener et al. (2004).

Chlorophyll molecules attached to PsaL and PsaM (Fig. 7A green arrows) have direct connection with others chlorophylls from the neighbor monomers located at interface area (Fig. 7B). Moreover, the close distance between these chlorophylls and those of neighbor monomers enable them to share the energy transfer network with the neighbor monomers rather than their own monomeric complex (Fig. 7B and 7C). Concerning chlorophyll molecules bound to PsaM has no energy connection with the same bound monomeric complex (Fig. 7A, 7B and 7C). These results explain why monomerization is always accompanied with presence of uncoupled

chlorophyll (El-Mohsnawy et al., 2010). Green arrow (3) bound to PsaK is considered the energy bridge transfer for chlorophylls bound to PsaM. Orange arrows that refer to carotenoids bound to PsaL and PsaB are considered the most likely stabilizers molecules in trimerization of PSI. This suggestion may explain the presence of monomeric PSI only in case of Δ PsaL mutant (Chitnis & Chitnis, 1993 and Klodawska et al., 2015), where both bound chlorophyll and carotenoids molecules are missing too.

Suggested splitting of trimeric PSI mechanism is shown in Fig. 8. Removing β-DM is an important step for safety of PSI complex, where the presence of detergent with organic solvents leads to damage of photosynthetic complexes (data not shown). As a result of suspending PSI trimers with hexane-DMSO mixture, the organic solvent leaks out through monomer-monomer interface area. Since hexane is non-polar solvent, it is able to eliminating large carotenoid molecules and partial chlorophyll molecules, which explains the emission of uncoupled chlorophylls at 679nm. Low DMSO concentration in hexane was efficient reagent for liberation the chlorophyll molecules located at monomer-monomer interface area leading finally to splitting of trimeric complex.

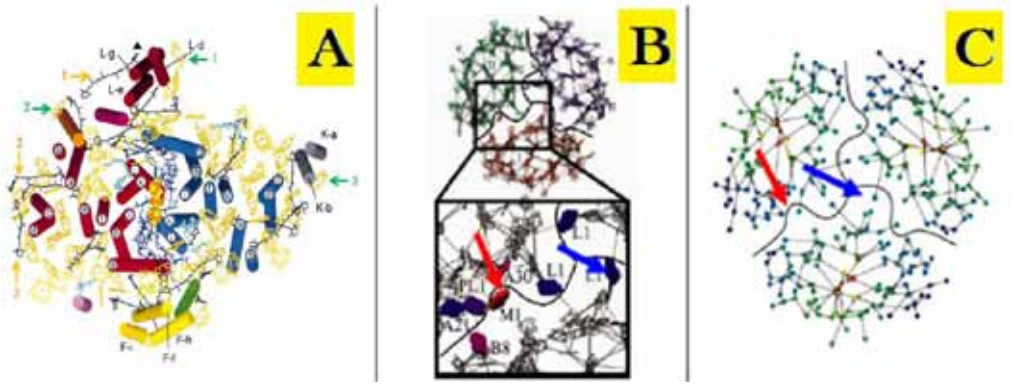

Fig. 7. A: Arrangement of cofactors and protein subunits within PSI (Fromme et al., 2001) [Green arrows refer to Chls bound to PsaL, PsaM and PsaK. Orange arrows refer to carotenoids bound to PsaL and PsaB], B: Zoom in view of the chlorophyll connections between neighboring monomers of PsaL and PsaM [Only the highest excitation transfer rates are indicated (Sener et al., 2004)] and C: Excitation migration [Red arrow points to Chl attached to PsaM and blue arrow points to the Chl *a* attached to PsaL (Sener et al., 2004)].

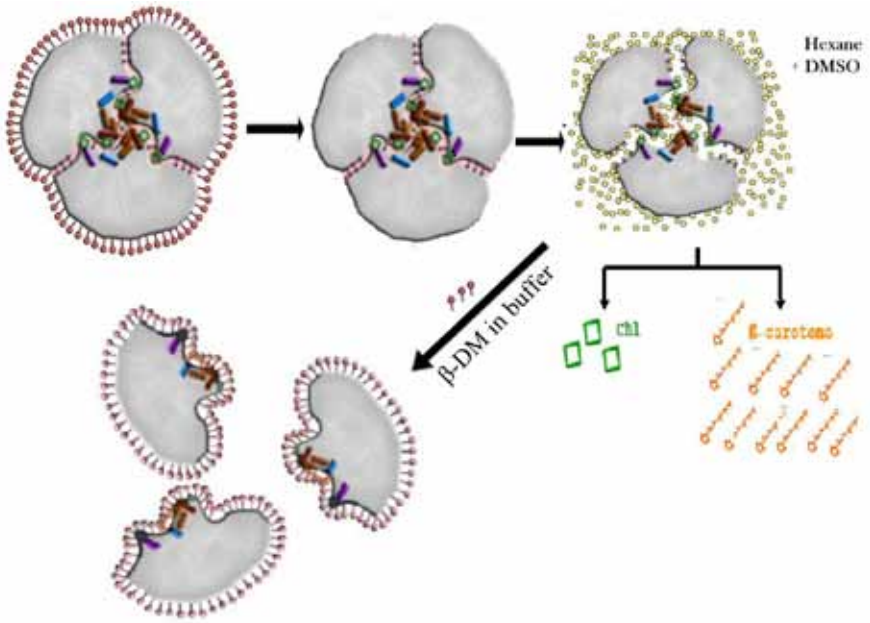

Fig. 8. Suggested diagram showing the role of chlorophylls and carotenoids located at monomer-monomer interface area on stabilization of the trimeric PSI of *Thermosynechococcus elongatus* (This figure is modified to that published by El-Mohsnawy et al., 2010).

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استقرار النظام الضوئى الثلاثى داخل ثيرموسينيكوكوكس الونجاتس عبر جزيئات كلوروفيل أ والكاروتينويدات الواقعة فى منطقة المواجهة للنظام الضوئى 1 المفرد

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من المسلم به تواجد الجزىء المفرد من النظام الضوئى 1 داخل الطحلب الأخضر المزرق ثيرموسينسكوكوس الونجاتس فى حالة التعرض إلى نقص شديد فى تركيز الحديد أو ازالة الوحدة (PsaL). تم عزل النظام الضوئى 1 بصورة أحادية عن طريق معالجة النظام الضوئى الثلاثى الموجود فى غشاء الثيلاكويد بتركيزات عالية من الأملاح فى ظل ارتفاع فى درجة الحرارة (50 درجة مئوية).

من خلال الدراسة الحالية يتم اذابة انتقائية للكاروتينويدات وكلوروفيل أ الموجود فى المنطقة المواجهة بين جزيئات النظام الضوئى الأحادى بواسطة خليط من الهكسان مع عدة مذيبات عضوية. وقد أظهرت النتائج أن خليط هكسان مع ثنائى المثيل اكسيد الكبريت أظهر أفضل نتائج فى شطر النظام الضوئى 1 الثلاثى إلى أحادى. وقد أكدت النتائج من خلال التدرج السكرى للسكروز والجل الأصلى الأزرق أن لكلورفيل أ والكاروتينويدات الموجودة فى الأوجة المتقابلة دور رئيسى فى عملية البلمرة. ومن جهة أخرى فقد أظهرت نتائج القياسات الوميضية الدقيقة عند درجة حرارة 77 كالفين وجود جزيئات كلوروفيل غير متصلة وازاحة فى قمة الوميض فى اتجاة الضوء الأزرق مما يدل على شطر النظام الضوئى الثلاثى. و قد أظهرت النتائج أن كفاءة المذيبات العضوية المستخدمة على نزع للكاروتينويدات أعلى من الكلوروفيل، حيث تم نزع 12 جزىء كلوروفيل من اجمالى 96 فى حين تم نزع حوالى 12 جزىء كاروتينويد من إجمالى 22 جزىء.