Modifications in photosystem 1 induced by iron deficiency in *Thermosynechococcus elongatus*

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Abstract: Iron is considered one of the most essential elements required by all organisms. The vital role of iron for photosynthetic organisms including cyanobacteria is its involvement as cofactor in a lot of enzymes, photosynthetic complexes and electron transport carriers. Photosystem 1 in *Thermosynechococcus elongatus* is existed in trimeric form that contains 12 Fe atoms / monomer. The production of IsiA (Iron starvations induced protein A) due to iron deficiency was accompanied with several hypotheses for its function. The adopted manuscript investigates the exact function of this supercomplex. The use of room temperature absorption spectra and 77K fluorescence emission and excitation spectral analysis of native and purified PS1-IsiA supercomplex exhibited existing of ß-carotene molecules within the investigated supercomplex. The combination of chromatographic fractionation with spectroscopical analysis proved monomerization of some trimeric photosystem 1 complexes. In addition, the ability of phycobilins to energy transfers to the bulk chlorophylls of photosystem 1 was not affected by IsiA protein. Activity measurements using O_2-consumption techniques showed low O_2 consumption of PS1-IsiA supercomplex compared to trimeric PS1, moreover, it was remarkably enhanced by increasing the light intensity. Finally, it could be concluded that *Thermosynechococcus elongatus* cells synthesis chlorophyll containing IsiA ring around PS1 to act as screen that absorbs the incident light to photosystem 1, hence the rate of electron flow is reduced. This ring contains ß-carotene molecules that dissipate energy from ring chlorophylls and consequently save the structure of photosystem 1 from damage under iron limitation conditions.
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1.Introduction

Cyanobacteria have large genetic pool those encode biochemical pathways to achieve optimal light utilization and avoid damage caused by excessive light (photoinhibition). For that reason, iron is considered an essential element for all organisms (Archibald, 1983). Despite existing of iron in high amount on earth, its biological availability is limited (Frausto da Silva and Williams, 1993; Lippard and Berg, 1994). Iron is considered the key of photosynthetic and respiratory electron transport chains. In addition, it acts as ion cofactor of ROS-detoxifying enzymes like catalase, peroxidase, and superoxide dismutase. Iron atoms are involved the structure of most proteins of photosynthetic electron transport chain (Ferreira and Straus, 1994). In PS1, there are three 4Fe-4S centers (Fx, FA and FB) and Ferredoxin has two iron atoms in the 2Fe-2S centre, in addition to one atom in cytochrome c_{553} (Jordan *et al.,* 2001 and Fromme *et al.,* 2001). PS2 contains a non-heme iron between QA, quinone that is bound to D2, and QB, the plastoquinone bound to D1 (Frerra *et al.,* 2004). The cytochrome *b6f* complex comprises five iron atoms which are bound in two hemes of cytochrome *b*6, one heme in cytochrome *f* and of two iron atoms within the 2Fe-2S centre of the Rieske protein (Whitelegge *et al.,* 2002; and Kurisu *et al.,* 2003). For this reason, iron deficiency leads to disturbance in electron transport chain as well as damage of the photosystems, expecially PS2 (Aro *et al.,* 1993; Bhaya *et al.,* 2000). On the other hand, high iron concentrations could be deleterious (Elstner, 1990). Since heme oxygenase enzyme catalyzes the first committed step of phycobilins biosynthesis, the amount of phycobilins is remarkably affected by iron limitation (Rhie and Beale, 1995).

Iron deficiency response is usually visualized as a large decrease in iron-containing molecules and those whose synthesis is dependent directly or indirectly on iron. Iron deficiency directly causes a degradation of cellular pigments, especially of chlorophyll-containing proteins and phycobilisomes as shown for *Synechococcus elongatus* PCC 7942 and *Synechocystis sp.* PCC 6803 (Hardie *et al.,* 1983; Sherman and Sherman, 1983; Riethman and Sherman, 1988). On the other hand, it leads to inducing proteins (IdiA, IsiA and IsiB) that regulate the photosynthetic electron transport chain (Straus, 1994; Dong and Xu, 2009). IdiA is assumed to protect the acceptor side of PS2 by interacting with the non-heme iron located between QA and QB, which acts as an iron storage during the turn-over of PsbA (Bagchi *et al.,* 2003;

Michel and Pistorius, 2004; and Arteni *et al.,* 2005). In contrast, IsiB, flavodoxin, replaces the iron-rich soluble electron transfer protein ferredoxin (Sandmann, 1985; Falk *et al.,* 1995). IsiA, antenna rings containing chlorophyll a around PS1-trimers, reduces the capacity for state transitions and consequently affects on electron transport chain (Ivanov *et al.,* 2000; Boekema *et al.,* 2001; and Nield *et al.,* 2003).

Concerning publications on IsiA suggested several functions; i.e.) protective role, where IsiA function as energy dissipation (Park *et al.,* 1999; Sandström *et al.,* 2001), increase the light harvesting capacity of PSI (Sherman and Sherman, 1983) or chlorophyll storage protein (Burnap *et al.,* 1993). Nield *et al.,* 2003 adopted a model for IsiA-PS1 supercomplex based on electron microscope data. According to their suggestion, IsiA is a ring composed of 18 protein subunits. Each one contains 12-13 chlorophyll molecules which act energy providers for PS1.

This manuscript acts to characterize the spectroscopical differences of PS1-IsiA and PS1 and also, the exact function of IsiA supercomplex.

2.Material and methods:

Cultivation conditions

Cultivation of *Thermosynechococcus elongatus* cells were achieved according to Ivanov *et al.* (2006) with some modifications. Preculture was prepared by cultivation of cells in liquid BG-11 (Rippca *et al.*, 1979) in rod-shaped glass tubes bubbled with 5-7 % CO_2 in air, 50 °C and continuous illumination of 50 $mmol.m^{-2}.s^{-2}$. Bi-distal water was used to prepare both normal BG-11 medium and that free of iron. Preculture was washed three times with BG-11 free iron media. Inoculums were injected to both normal and BG-11 free iron media to reach OD_{750nm} of 0.2. Cultivation stopped when the OD_{673nm} was higher than OD_{680nm}.

Absorption spectral analysis

Absorption spectra of 0.5 ml of culture or purified complexes samples were measured by Shimadzu UV-2450.

77K fluorescence emission and excitation analysis.

Fluorescence emission spectra were performed according to Schlodder *et al.* (2007) using SLM-AMINCO Bauman, Series 2 Luminescence spectrometer. Samples were diluted to a chlorophyll concentration of 8 µg/ml in buffer containing (20 mM HEPES pH 7.5, 10 mM $MgCl_2$, 10 mM $CaCl_2$ and 60 % glycerol) 0.02 % ß-DM was added in case of purified PS1 or PS1-IsiA supercomplex. The diluted samples were frozen to 77 K by gradually immersing in liquid nitrogen. 440, 500 and 580 nm actinic light were used for excitation. Fluorescence emission spectra were measured from 600 to 800 nm with a step size of 1 nm and a bandpass filter of 4 nm. Excitation fluorescence spectra were measured in a range from 400 to 800 nm. It was measured with a step size of 1 nm and a bandpass filter of 4 nm at emission fluorescence band at 730 nm.

Purification of PS1-IsiA supercomplex and PS1.

PS1-IsiA and trimeric PS1 was purified from 10-his-PsaF mutant of *Thermosynechococcus elongatus* according to protocol of Boekema *et al.* (2001) and Prodöhl *et al.* (2004). The 1st purification step was achieved by IMAC column while the 2nd purification step was performed using IEC. Ion exchange column was equilibrated by 6 CV before loading the samples. PS1-IsiA supercomplex was eluted at 60 mM $MgSO_4$, while PS1 was eluted at 90 mM $MgSO_4$.

O_2 consumption estimation

The activity of purified PS1-IsiA and PS1 complexes were measured as O_2- consumption in response to illumination using the FIBOX 2 (PreSens) at 30 °C. After calibration, 3 µg chlorophyll containing samples were mixed with buffer pH 7.5 (30 mM HEPES, 3 mM $MgCl_2$, 50 mM KCl, 330 mM mannitol and 0.03 % ß-DM) to a final volume of 1 ml in the presence of 80 µM DCPIP and 50 µM Na-ascorbate as electron donor and The reagents were stirred in dark for 2 min followed by illumination by 1500, 2500 µE $m^{-1}s^{-1}$ white light. The average rate of O_2-uptake before illumination was subtracted from that after illumination. The activity was calculated using the following equation:

Activity = ΔSx60x1000/Cx1000 = µMol O_2 $mg\text{-}Chl^{-1}$ h^{-1}

ΔS difference, C Chlorophyll concentrations µg

3.Results:

Since iron is an essential element and present heavily in cell and thylakoid membranes of *T. elongatus*, it plays an important role in both respiratory and photosynthetic processes. Hence, its starvation effects on various metabolic activities were monitored by using spectroscopical analysis. The room temperature absorption spectral comparison between normal and free deficiency media are shown in Figure 1A. Blue-shift and minor reduction were observed at 680 nm in case of stressed cells. In addition, a huge reduction in phycocyanin and allophycocyanin were monitored by decreasing the absorbance at the range 620-650 nm. In normal cells, the absorbance at 620 nm was higher than 680 nm, these results obviously reversed in case of Fe stressed cells. This reduction extended to reach the ß-carotene at 460 to 510 nm. Figure 1B summarizes reduction in absorbance at different wavelengths, where all wavelengths showed reduction and the highest reduction was detected at 730 nm (far-red chlorophyll region). In contrast, high absorbance was observed at

280 nm, which indicated high protein synthesis. Fluorescence emission spectral changes at 77K are shown in Figure 2. The use of different actinic light exhibited almost the same spectral shifts, while the intensities were extremely different. By using 440 nm actinic light, two remarkable variations were observed. Very high fluorescence emission at 686 nm and quite low fluorescence at 732 nm were detected. In addition, 6 nm-blue shifts from 732 nm to 726 nm were observed in case of stressed cells. In addition to small blue-shift at 663 nm to 661 nm, almost the same results were obtained in case of using 500 nm actinic light (Figure 2B). In contrast, 77K fluorescence emission spectra of Fe-stressed cells using 580 nm actinic light exhibited also a lot of variations compared cells grown in normal medium. As shown in figure 3C, 6 nm blue-shifts were detected in case of Fe-stressed cells with low emission value at far-red region (732 nm to 726 nm), while 2 nm red-shifts with high emission value were observed at red region (684 nm to 686 nm). The fluorescence excitation spectra of Fe-stressed cells at 730 nm emission showed interested results (Figure 3). Reduction excitation fluorescence values at 460-510 nm, 683 nm were detected. In addition, a shoulder at 673 nm was enhanced to be a main peak due to iron deficiency condition. Excitation at 620 and 650 nm did not show remarkable changes. Figure 4 shows that, not all PS1 are associated with IsiA super-complex in *Thermosynechococcus elongatus* cells exposed to iron deficiency, where 2 detectable peaks at 60 and 90 mM $MgSO_4$ were obtained. It should be announced that the peak at 60 mM $MgSO_4$ that expressed to PS1-IsiA super-complex was larger than that eluted at 90 mM $MgSO_4$, which indicates how much PS1-IsiA super-complex was. Astonishing absorption spectra were observed by purified PS1-IsiA super-complex, where remarkable absorbance enhancement at carotene region (500 nm) was observed that indicated increasing in ß-carotene. In contrast, remarkable absorbance depletion was recorded after 710 nm, which indicates low far-red chlorophylls (Figures 5 A, B). On the other hand, the 77K fluorescence emission spectral analysis of purified PS1-IsiA super-complex exhibited 6 nm blue-shifts compared to purified trimeric PS1. Also, high peak at 686 nm was observed in case of illumination by 440 nm. This peak was remarkably reduced in case of illuminating by 500 nm (Figure 6). To evaluate the role of IsiA complex either in activation or inhibition of PS1 activity, oxygen consumption measurements have been examined using two different light intensities (Table 1). In both cases, the purified PS1-IsiA exhibited low O_2-consumption compared to purified PS1 trimer that recorded 41 % and 47 % for **1500** $\mu E*m^{-1}*S^{-1}$ and **2500** $\mu E*m^{-1}*S^{-1}$, respectively. These results may indicate that IsiA ring might screen the incident light to bulk chlorophyll of PS1. It could be concluded that the spectroscopical analysis exhibited a lot of variations in the maximum absorbance and fluorescence of chlorophyll and phycobilins as well as some blue and red shifts in case of complete cells. Concerning purified IsiA-PS1 super complex, absorption spectra showed more ß-carotene and less far-red chlorophyll compared to purified PS1, while the fluorescence spectra proved connection between IsiA-ring containing chlorophylls with ß-carotene. Activity measurements for purified IsiA-PS1 supercomplex exhibited low activity at low light intensity, which enhanced by increasing the light intensity.

Table 1: Activity measurements based on O_2 consumption of purified PS1 and PS1-IsiA supercomplex. Activity was expressed as µMol O_2 $mg\text{-}Chl^{-1}\ h^{-1}$

	Activity at 1500 $\mu E*m^{-1}*S^{-1}$	Activity at2500 $\mu E*m^{-1}*S^{-1}$
Purified PS1	791.5 ± 10.25	1076.3 ± 12.69
PS1-IsiA	303.8 ± 8.5	504.5 ± 9.33
%Activity of PS1-IsiA	40.9 %	46.87 %

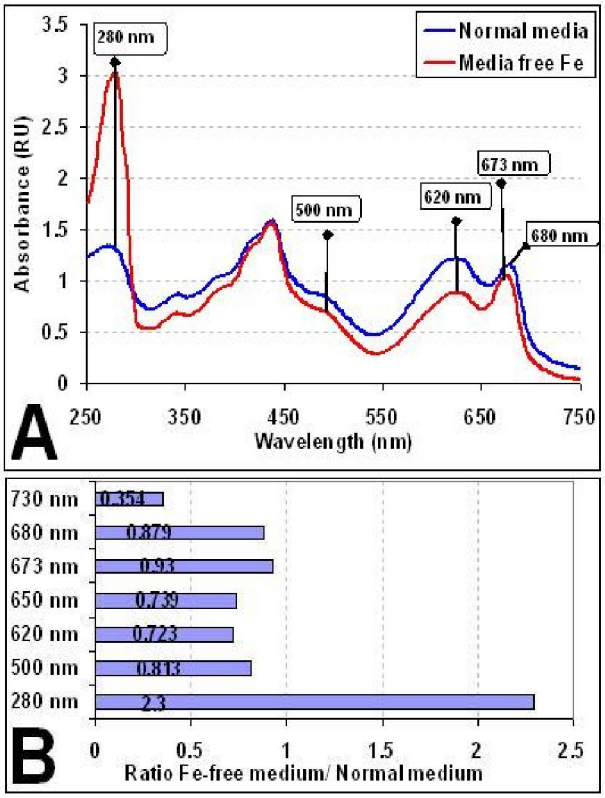

Figure 1: A. Absorbance spectra of *T. elongatus* cells grown in normal and iron deficiency media. B. Ratio Fe-free medium to normal medium at specific wavelengths.

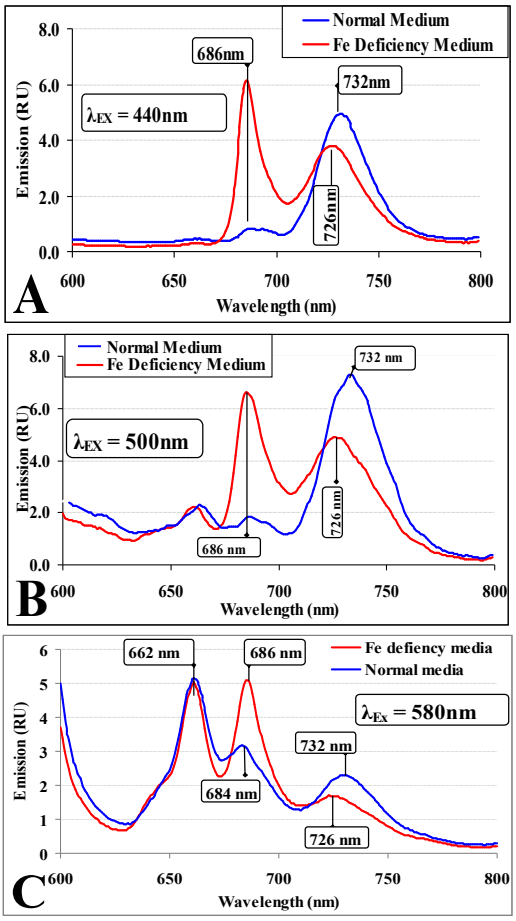

Figure 2: 77K Fluorescence emission spectra of *T. elongatus* cells grown in normal and iron deficiency media. Cells were diluted with buffer containing 20 mM HEPES, 10 mM $CaCl_2$, $MgCl_2$ and 60% glycerol to reach 8µg Chl/ml. Samples were gradually frozen by liquid nitrogen before measurements. A. the used actinic light was 440 nm, B. the used actinic light was 500 nm and C. the used actinic light was 580 nm.

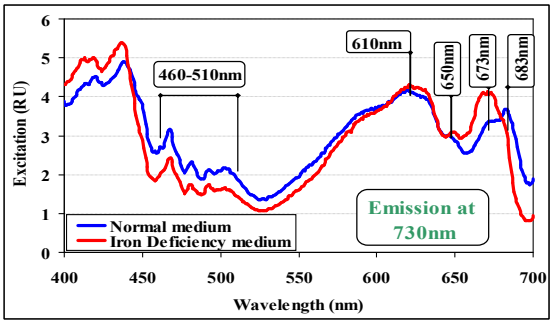

Figure 3: Fluorescence excitation spectral analysis of *T. elongatus* cells grown in normal and iron deficiency media. Cells were diluted with buffer containing 20 mM HEPES, 10 mM $CaCl_2$, $MgCl_2$ and 60% glycerol to reach 8µg Chl/ml. Samples were gradually frozen by liquid nitrogen before measurements. Emission was observed at 730 nm.

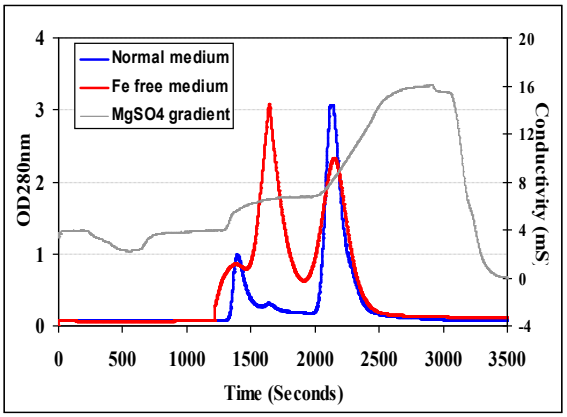

Figure 4: Elution profile of purified PS1 and PS1-IsiA. POROS HQ column was equilibrated by 5 CV of MES pH 6.5 buffer (20mM MES, 10 mM $MgCl_2$, 10 mM $CaCl_2$, 0.03% ß-DM) before loading samples. A leaner gradient 0-200 mM with step at 60 mM $MgSO_4$ were applied for elution both PS1-IsiA Super-complex and trimeric PS1 at 60 mM $MgSO_4$ at 90 mM $MgSO_4$, respectively.

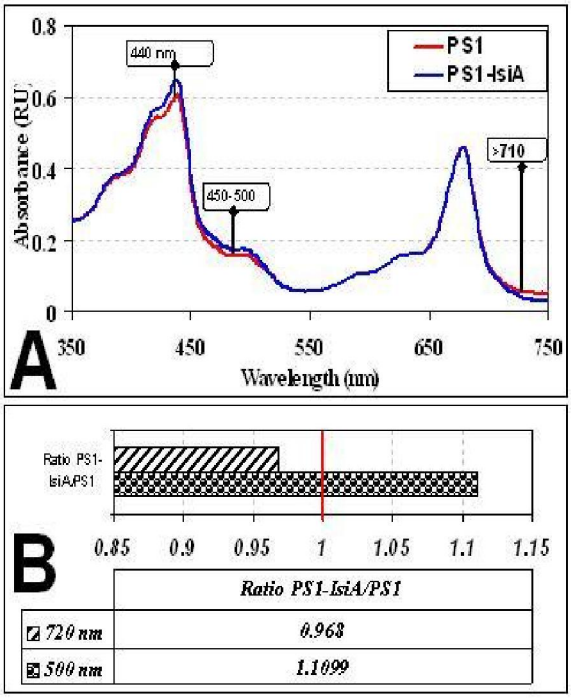

Figure 5: A. Absorption spectra of purified PS1-IsiA and PS1 isolated from *T. elongatus*. B. The ratio PS1-IsiA to PS1.

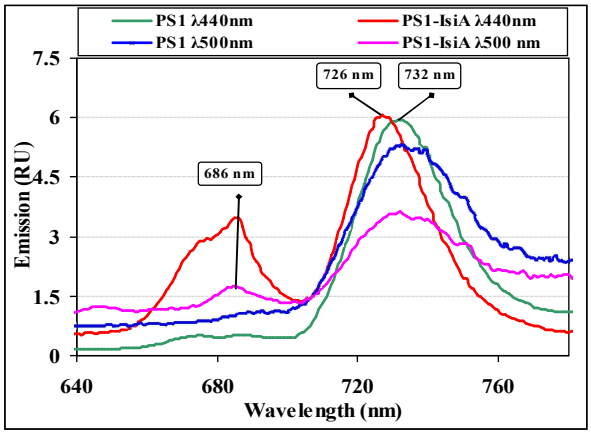

Figure 6: 77K Fluorescence emission spectra of purified IsiA-PS1 and PS1 from *T. elongatus*. Samples were diluted with buffer containing 20 mM HEPES, 10 mM $CaCl_2$, $MgCl_2$ and 60% glycerol to reach 8µg Chl/ml. Samples were gradually frozen by liquid nitrogen before measurements. The used actinic lights were 440nm and 500 nm

4.Discussion:

Iron has a unique role in the photosynthetic electron transport chain, where 23 to 24 iron atoms are involved in the photosynthetic electron transport (Ferreira and Straus, 1994). The main function of IsiA supercomplex is still under debate (Boekema *et al.,* 2001; Kouril *et al.,* 2005). The presented results try to show and explain the changes in PS1 complexes due to iron deficiency as well as the function of this supercomplex. The shifts in the maximum bulk absorption spectra from 680 nm to 673 nm and the depletion of absorbance of purified PS1-IsiA supercomplex at wavelengths more than 710 nm should be due to monomerization of some trimeric PS1 (El-Mohsnawy *et al.,* 2010). These results were supported by chromatographic fractionation of PS1 and PS1-IsiA from iron deficiency cells and came in agreement with suggestion of Ivanov *et al.* (2006), who pointed to monomerization of trimeric PS1 due to iron deficiency and suggested binding of the liberated chlorophyll to IsiA protein or be dissociated. This suggestion is supported by results published by Boekema *et al.* (2001) for mesophilic cyanobacterium *Synechococcus sp.*, where the amount of trimeric PS1 was reduced by 80% under iron deficiency. The fluorescence emission spectra of *T. elongatus* cells by 440 nm and 500 nm actinic showed two main chlorophyll bulks in PS1-IsiA super complex at 728 nm and the 2nd one was at 686 nm, which was quit high compared to shift published by Andrizhiyevskaya *et al.* (2002). This result proved also the monomerization of trimeric PS1 (Ivanov *et al.*, 2006; El-Mohsnawy *et al.,* 2010). The most important and new results have been observed in case of illumination by 500 nm actinic light for IsiA in thylakoid membrane or purified one, where the same fluorescence emission behavior as in case of excitation by 400 nm was detected. This result prove the connection of ß-carotene with IsiA chlorophyll molecules, hence the previous suggested models of Boekema *et al.* (2001) and Nield *et al.* (2003) may need to be modified. These results came in agreement with that of room temperature absorption, which exhibited high absorbance in carotenoids regions in purified PS1-IsiA super complex. On the same approach came the fluorescence spectra using 580 nm actinic light. These data indicated that phycobilins should be still connected with PS1 in addition to IsiA supercomplex (Rakhimberdieva *et al.,* 2004).

An obvious picture was monitored through excitation spectra of cells exposed to iron deficiency. Presence of excitation peak at 673 nm indicates the connection of chlorophyll molecules bind to bulk chlorophyll of PS1, while almost the same value was detected at 620 nm and 650 nm, which indicates high efficient binding of phycobilins to the bulk chlorophyll of PS1 even under phycobilins degradations (Rhie and Beale, 1995). These results gainsay the suggestion of Burnap *et al.* (1993) and prove energy flow from phycobilins to PS1. On the other hand, the combination of low excitation fluorescence at carotenoids region (460-510 nm) with absorbance of purified PS1-IsiA supercomplex gave additional strong evidence to existing of ß-carotene molecules within IsiA ring. This hypothesis is supported by fluorescence emission spectra of purified PS1-IsiA super complex, where a detectable peak at 686 nm was observed in case of illumination by 500 nm actinic light. The function of IsiA supercomplex should be optical masks around PS1 that absorb incident light protecting it from photo-damage due to absence of iron ions and stopping the electrons flow, while carotenoid molecules bound to IsiA ring induced non-photochemical fluorescence quenching for energy dissipation (Rakhimberdieva *et al.*, 2004, 2010). This suggestion was supported by activity measurements of purified PS1-IsiA supercomplex compared to purified PS1. Low oxygen consumption of the supercomplex, which enhanced by increasing the light intensity proved to low energy transport efficiency between IsiA supercomplex to PS1 reaction center. Moreover, this supercomplex may screen the light intensity to the bulk chlorophyll.

It could be concluded that *Thermosynechococcus elongatus* cells act to resist iron deficiency by monimerizing some of trimeric PS1 content and cover it by IsiA ring, which acts as a screen that minimized the incident light to PS1. This chlorophyll containing ring should have addition ß-carotene molecules which

dissipate the energy. This ring should not enhance the energy transfer to PS1.

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