Chapter 8

A Photo-Protective Role for IsiA in the Early Stage of Iron Deficiency and at Cryogenic Temperatures

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Cyanobacteria live in water habitats, where they are subject to a great scala of environmental fluctuations. One of the possible fluctuations concerns the local iron-concentration. In a response to iron deficiency, the \textit{isiA} gene of the cyanobacteria expresses the production of a pigment-protein complex, known as IsiA or CP43'. It has been shown that long-term iron deficiency results in the formation of large IsiA-aggregates, and that some of these aggregates associate with photosystem I (PSI) while others are not connected to a photosystem. The unconnected complexes give rise to a strong fluorescence peaking at 687 nm at 4 K, which is strongly quenched at room temperature on an average time scale of 130 ps (1). In this study, we report a time-resolved fluorescence study of IsiA-aggregates at low temperatures. The average time scale of fluorescence quenching is estimated to be about 600 ps at 5 K and 150 ps at 80 K. Both lifetimes are much shorter than that of the monomeric complex CP47, which has an average lifetime of about 5 ns at 77 K. We conclude that IsiA-aggregates quench fluorescence to a significant extent already at cryogenic temperatures. We also show by low-temperature fluorescence spectroscopy that unconnected IsiA-aggregates are present already after two days of iron deficiency and that under these conditions the fluorescence quenching is similar to that after 18 days, when PSI is completely absent. We conclude that unconnected IsiA provides photoprotection in all stages of iron deficiency.
Introduction

Cyanobacteria are responsible for a significant part of oxygen and biomass production on earth. Cyanobacteria live in water habitats, where they are subject to great variability of the environmental conditions. One of the possible fluctuations concerns the local iron concentration. In a response to iron deficiency, the \textit{isiA} and \textit{isiB} genes of the cyanobacteria express the production of respectively IsiA (also called CP43') and flavodoxin (2,3). The latter will replace the iron-containing ferrodoxin associated with photosystem I (PSI), responsible for electron transport (4). IsiA is a pigment-protein complex from the family of core antenna complexes and shows great similarity with the CP43 core antenna complex of PSII (5). From pigment analysis, IsiA was found to bind chlorophyll (Chl) $a$, $\beta$-carotene, zeaxanthin and echinenone (1).

It has been shown that IsiA can form large ring structures around PSI, the number of copies and rings depending on the duration of iron deficiency (6). In the early iron depleted stage, PSI-IsiA complexes are predominantly found to consist of a trimeric PSI core, surrounded by a ring of 18 copies of IsiA (PSI$_3$IsiA$_{18}$) (7,8,9). In long-term iron deficiency conditions, IsiA is found to form rings around PSI monomers as well. After pursued iron deficiency, IsiA was also found to form ring structure aggregates devoid of PSI (1,6). Flexibility of the number of copies that can be found in IsiA ring structures has also become evident in work with a \textit{psaFJ} less mutant in the absence of iron limitation (10).

Primarily, the association of IsiA enlarges the PSI absorption cross-section. The increased antenna size will compensate for the lower amount of PSI in the cyanobacteria grown in the absence of iron, because of its high dependence on iron (3,4). Photosystem II (PSII) on the other hand, is less influenced by the absence of iron and there is no evidence for binding of IsiA with PSII. Thus, the electron transfer among the two photosystems is equilibrated by the increase of the PSI light-harvesting antenna by binding of IsiA. In the PSI$_3$IsiA$_{18}$ supercomplex, the IsiA antenna was shown to lead to a blue-shifted excited state distribution and lengthening of the excitation energy trapping time by a factor of about two with respect to the PSI core complex (i.e. from about 20 ps to 40 ps) (11,12). The increase of the trapping time was interpreted in both studies to be a result of efficient energy transfer from IsiA to PSI (11,12). A fast intrinsic rate constant of energy transfer from IsiA to PSI of (2 ps)$^{-1}$ was required to explain the kinetics in PSI-IsiA. According to Förster calculations, 2 ps is equivalent to an interpigment distance of 13-14 Å between IsiA and PSI. However, this distance was predicted to
be 22-33 Å from the structural model (13). The experimental results suggest the presence of undiscovered linker pigments between IsiA and the PSI core, which would moreover explain the discrepancy of the high number of Chls (16-17) estimated for IsiA in PSI$_3$IsiA$_{18}$ complexes by Andrizhiyevskaya and co-workers (12) with respect to the known 13 for CP43 (14).

The presence of IsiA-aggregates lacking PSI is, however, suggested to serve a photo-protective role. Melkozernov and co-workers (15) agree with the light-harvesting role of IsiA for PSI, but argue that the IsiA-aggregates and IsiA-PSI supercomplexes with more IsiA than the PSI$_3$IsiA$_{18}$ structure arise at unrealistically strong iron depletion and thus cannot be representative of the natural situation. In contrast, Ivanov and co-workers (16) measured a decrease of the effective absorption cross-section of PSI upon iron starvation in cyanobacterial cells in vivo and thus conclude that IsiA can only serve a photo-protective function. In their work, however, the emission at 687 nm was interpreted as fluorescence from PSII, whereas Andrizhiyevskaya and co-workers (17) had shown this peak to arise from IsiA. If PSI degrades there is need for the electron transport from PSII to slow down, thus IsiA-aggregates dissipate energy which would otherwise result in electron flow from PSII (6). Energy dissipation into heat leads to a decrease in fluorescence yield and is known as non-photochemical quenching (NPQ). For example, NPQ is the protective mechanism permanently exploited by evergreens under freezing conditions (18,19). More well-known are NPQ mechanisms that quench excitation energy at high light intensities only, both in green plants (20) and in cyanobacteria (21,22).

IsiA-aggregates were shown to strongly quench excitation energy by ultrafast fluorescence measurements (1). Time-resolved fluorescence measurements revealed an average quenching time of 130 ps at room temperature, which is the fastest quenching time known thusfar to occur in native photosynthetic complexes. In this study we show time-resolved fluorescence measurements of IsiA-aggregates at low temperatures. Analysis of the emission decays reveal to which extent quenching occurs at these temperatures. We also aimed to find out whether IsiA serves to harvest light for PSI and/or has a photo-protective function in early stages of iron depletion. To this end, we measured temperature dependent emission from cells as a function of time since iron depletion.
Materials and methods

Organism and culture

Wild-type *Synechocystis* sp. strain PCC 6803 was grown at 30 °C in liquid culture in BG11 mineral medium at a light intensity of 50 μmol of photons m\(^{-2}\)s\(^{-1}\) in ambient air. Iron deficiency was achieved by omitting all iron sources in the medium. Inoculation for iron depleted culture was by 20-30 fold dilution of cells that were pre-grown in normal medium. In the work presented here, cells were harvested 0-18 days after inoculation. IsiA-aggregates were isolated as described in (1) from cells harvested 40 days after inoculation.

Steady-state spectroscopy

Samples were diluted in 20 mM BisTris pH 6.5, 5 mM MgCl\(_2\), 0.03% β-DM and 60% v/v glycerol to an OD at 680 nm of ~0.05 cm\(^{-1}\). A 4 K Utrex Cryostat with a gas-flow temperature controller was used to vary the temperature between 4 and 240 K. Absorbance measurements at 5 K were assessed on a laboratory-built spectrometer. Fluorescence emission spectra were recorded using a laboratory-built setup as well, equipped with a 0.5 m imaging spectrograph and a CCD camera (Chromex Chromcam I). Broadband excitation was provided by a tungsten halogen lamp (Oriel) and a 420 nm interference filter (bandwidth ~15 nm). The spectral resolution was 0.3 nm. The recorded emission spectra were corrected for the wavelength sensitivity of the detection system.

Time-resolved emission

Time-resolved emission was recorded using a streak camera setup. Samples were diluted in 20 mM BisTris pH 6.5, 20 mM NaCl, 0.06% β-DM and 66% v/v glycerol to an OD at 680 nm of < 0.1 cm\(^{-1}\). The sample was placed in a 1 cm pathlength polystyrene cuvette in the 4K Utrex Cryostat. The temperature was gas-flow controlled. Vertical excitation pulses of 400 nm (100-200 fs) were generated using the frequency doubled output of a titanium:sapphire laser (Coherent, VITESSE) with a regenerative amplifier (Coherent, REGA). The repetition rate was 25 kHz, pulse energy 40 nJ and the beam diameter ~1 mm. The fluorescence was detected at right angle through an orange sharp cut-off filter and polarizer at magic angle by a Chromex 250IS spectrograph, a Hamamatsu 5680 synchroscan streak camera and a cooled Hamamatsu C4880 CCD camera. Measurements were performed on a 2 ns time base with a time resolution of 27 ps.
Results and discussion

Low temperature time-resolved fluorescence of IsiA-aggregates

Time-resolved fluorescence has been measured of isolated IsiA-aggregates of *Synechocystis* sp. PCC 6803 after 40 days of iron depletion at temperatures in the range from 5 to 230 K upon 400 nm excitation. Time-resolved fluorescence at room temperature of the same material was reported earlier by Ihalainen and co-workers (1). The experiments at lower temperatures offer the opportunity to reveal the role of excitation energy quenching in IsiA at low temperatures.

The recorded fluorescence decays could be well fit with a four-exponential, irreversible, sequential decay at all temperatures according to the model of Figure 1 (see (23,24) for reviews on time-resolved analysis). Thus, upon excitation, the IsiA-aggregates evolve through four different emitting states (1-4), which have different lifetimes ($\tau_{1-4}$). The emission spectra of these intermediate states are shown for all temperatures in Figure 2, the so-called Species Associated Spectra (SAS). It should be stressed that the various SAS, which result from the modeling in Figure 1 at temperatures over the range from 5 to 230 K, can not be directly related to each other. For that purpose, a more extended target analysis is required (23,24), in which a kinetic model is established to describe the data. In the current analysis, we wish to focus on the development of the excitation energy trapping time scales with temperature. The relative amount of excitations lost from each intermediate emitting state (x) is expressed by $A_x$ in Figure 1 and summarized in Table 1 together with the different decay times associated with each state for all temperatures. The results of the experiments at room temperature (1) are added to Table 1.

It is clear that upon excitation, the high energy pigments – the ones with most blue absorption wavelengths – will transfer their excitation energy to nearby pigments which have their absorption wavelength more to the red and thus have lower electronic energy. In this way, the excitation energy is transported around the system until it meets one of the traps present in the aggregate. The density of traps, their energy level and the trapping mechanism will determine the rate of excitation energy quenching. If the trap involves pigments with rather blue absorption wavelengths, it will be difficult for more red absorbing pigments to transfer their excitation energy uphill into the trap. However, thermal energy can facilitate uphill energy transfer when it can compensate for the difference in energy between the pigments. Thus, upon lowering the temperature, the available thermal energy decreases and uphill energy transfer becomes less probable. In that case, the
excitations present in the IsiA-aggregate relax to the lowest energy level they can reach, that is the pigment with most red absorption wavelength, where they will stay during the pigments’ natural fluorescence lifetime.

At 5 K the four determined SAS according to the model of Figure 1, have emission maxima at 684, 686, 688 and 690 nm with respective decay times of 20, 200, 1300 and 6500 ps, see Figure 2. The first SAS with lifetime of 20 ps traps 18% of the initial excitations (Table 1). In the meantime, the excitations that are not trapped in 20 ps are transferred to pigments with more red absorption, as can be seen from the second resolved SAS with lifetime of 200 ps, which has a 2 nm red-shifted emission maximum with respect to the 20 ps SAS. We conclude that at 5 K trapping of excitations already occurs in the early time range. The same observation is made at 40, 80, 140 and 230 K, with respective trapping times of 10,

Fig 1. Simple kinetic model to describe the fluorescence recorded of IsiA-aggregates (isolated from Synechocystis sp. PCC 6803 grown 40 days without iron) as a unidirectional, sequential decay at different temperatures (5-230 K). Upon excitation, the IsiA-aggregates evolve through four emitting states (1-4), which each quench a fraction of the initial excitation population (A1-4) on different time scales (τ1-4). The data are summarized in Table 1.

<table>
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<th>(ps)</th>
<th>5 K</th>
<th>40 K</th>
<th>80 K</th>
<th>140 K</th>
<th>230 K</th>
<th>293 K</th>
</tr>
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<tr>
<td>τ1/A1</td>
<td>20/0.18</td>
<td>10/0.09</td>
<td>10/0.28</td>
<td>35/0.22</td>
<td>20/0.27</td>
<td>10/0.14</td>
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<tr>
<td>τ2/A2</td>
<td>200/0.34</td>
<td>150/0.46</td>
<td>110/0.43</td>
<td>100/0.55</td>
<td>50/0.44</td>
<td>66/0.48</td>
</tr>
<tr>
<td>τ3/A3</td>
<td>1300/0.39</td>
<td>650/0.36</td>
<td>400/0.24</td>
<td>350/0.20</td>
<td>150/0.28</td>
<td>210/0.37</td>
</tr>
<tr>
<td>τ4/A4</td>
<td>6500/0.09</td>
<td>5700/0.09</td>
<td>7200/0.05</td>
<td>12000/0.03</td>
<td>25000/0.01</td>
<td>1900/0.01</td>
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<td>τ5/A5</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>20000/0.01</td>
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<td>800</td>
<td>500</td>
<td>500</td>
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<td>130</td>
</tr>
<tr>
<td>SUM(τ1-3)</td>
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<td>300</td>
<td>150</td>
<td>130</td>
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<td>110</td>
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Table 1. Fluorescence decay time scales (τ) and relative loss of excitations (A) resulting from the analysis of the fluorescence decay of IsiA-aggregates (isolated from Synechocystis sp. PCC 6803 grown 40 days without iron) at different temperatures (5-230 K) according to the model in Figure 1. In the last column, the data of the similar study at room temperature are added as published in (Ihalainen 2005). Estimated error in the lifetimes is 10%.
Fig 2. SAS estimated from the fluorescence decay of IsiA-aggregates (isolated from *Synechocystis* sp. PCC 6803 grown 40 days without iron) according to the model of Figure 1 at the temperatures 5, 40, 80, 140 and 230K.
10, 35 and 20 ps. At 40 K the percentage of trapped excitations on the early time scale is the smallest, namely 9%. At 140 and 230 K, there is no red-shift among the SAS of the first two determined intermediate states (Figure 2). At room temperature, Ihalainen and co-workers (1) found a fast trapping component with lifetime of 9 ps. The resolved decay times in the early time range are seen to vary among the different temperatures. As mentioned before, the origin of either SAS can differ among the different temperatures.

For the second resolved SAS similar observations are made at 5, 40 and 80 K, where part of the excitation energy becomes quenched (Table 1) and the remaining fraction is transferred to pigments with a red-shifted emission spectrum (third SAS in Figure 2). At 140 K there is hardly any spectral difference between the SAS of the second and third determined intermediate state, whereas at 230 K, the second trapping phase is observed to have more red emission than the third (Figure 2). The dominant contribution to the trapping of excitation energy by this second phase at all temperatures becomes clear from Table 1, respectively 34, 46, 43, 55 and 44% of the excitations become quenched at 5, 40, 80, 140 and 230 K. The lifetime of the second SAS is observed to increase from 50 ps at 230 K to 200 ps upon cooling down to 5 K, probably because it becomes harder for excitations to reach this trap at decreasing temperature as mentioned earlier. Consequently, the emission maximum of this SAS is observed to significantly red-shift from 680 nm at 230 K to 686 nm at 5 K.

The third resolved SAS with respective lifetimes of 1100, 650, 400, 350 and 150 ps at temperatures of 5, 40, 80, 140 and 230 K do also quench a significant fraction of the excitation energy, respectively 39, 36, 24, 20 and 28% (Table 1).

In the long time range, a small excitation energy loss is found to occur with lifetimes of 6.5, 5.8, 7.2, 12 and 25 ns at the respective temperatures of 5, 40, 80, 140 and 230 K. The contribution of this phase is seen to decrease upon raising the temperature, from 9% at 5 K to 1% at 230 K and represents the relative amount of excitation energy which remains on the IsiA-aggregates during this long lifetime (Table 1). The SAS differ among the different temperatures, with maxima at 690, 692, 683.5, 681 and 680 nm for respectively 5, 40, 80, 140 and 230 K. The increase of the lifetime for temperatures above 80 K will be due to a lack of resolving power, since this contribution becomes very small and the measurements were performed on a time scale of 2 ns, which makes it difficult to resolve such long-lived components as can be seen from the offset of these spectra in Figure 2.

The final trapping state with emission maximum at 690 nm and lifetime of 6.5 ns (Figure 2) represents the fluorescence lifetime of IsiA-aggregates at 5 K and
is in good agreement with the fluorescence lifetime of CP47 as was measured in a similar experiment at 77 K to be 5.8 ns (25). The main trapping phases of the IsiA-aggregates at 5 K, however, have lifetimes of 0.2 and 1.3 ns (Table 1), which are significantly shorter than the 5.8 ns fluorescence lifetime of CP47. Upon increasing the temperature to 230 K, the lifetimes of the two main trapping phases are seen to decrease to respectively 0.05 and 0.15 ns. In Table 1, the overall fluorescence decay time (indicated as SUM ($\tau_{1-4}$)) is shown to decrease from 1.2 ns to 0.32 ns upon increasing the temperature from 5 to 230 K. Notice that the overall fluorescence decay time represents the fluorescence quantum yield. The average fluorescence quenching time can be calculated from the first three trapping phases (indicated as SUM ($\tau_{1-3}$)) to be 600 ps at 5 K. Since this is much faster than the 5.8 ns decay time resolved for CP47 at 77 K, we conclude the occurrence of fluorescence quenching in IsiA-aggregates even at 5 K. This average quenching time is seen to decrease upon increasing the temperature from 600 ps at 5 K to 70 ps at 230 K. The average quenching time at 230 K is clearly shorter than that estimated in a similar way for the IsiA-aggregates measured at room temperature in an earlier experiment, which is 110 ps (1) (Table 1). The difference is probably due to some variation in the growth of the *Synechocystis* sp. PCC 6803 cells and/or the isolation of the IsiA-aggregates.

**Low temperature absorption and fluorescence of *Synechocystis* cells as a function of iron depletion**

The strong quenching at all temperatures described above was measured in IsiA-aggregates isolated from cells grown for a very long time in the absence of iron. The question arises if the fluorescence properties of these aggregates reflect a natural situation and if IsiA formed in *Synechocystis* cells in much earlier stages of iron deficiency has similar quenching properties. For this reason we recorded low temperature steady-state absorption and emission spectra as a function of time since iron depletion for whole cells of *Synechocystis* sp. PCC 6803. In Figure 3 the absorption spectra at 5 K of *Synechocystis* cells grown without iron during 0-18 days are displayed. The spectra have been normalized to the optical density of the Chls in the Qy-region (665-700 nm). At day 0, main absorption bands are observed at 672, 683 and 691 nm. The maxima can safely be assigned to PSI (26), also because the content of PSI in wild-type cells is about 5-10 times higher than that of PSII. After 18 days of iron depletion, the cells show pronounced absorption bands at 670, 674.5 and 680 nm. We have shown before that IsiA is the predominant Chl binding complex after prolonged iron starvation (1,6), so we assign these
absorption bands to IsiA. The spectra of cells grown between 0 and 18 days without iron show that the spectra are virtually identical after 3 days of growth without iron, and that the change from mainly PSI absorption to mainly IsiA absorption occurs between 1 and 2 days of growth without iron.

Figure 4a shows the 5 K emission spectra upon 420 nm excitation of cells of *Synechocystis* sp. PCC 6803 grown without iron for 0-18 days. The spectra have been normalized to the Chl *a* absorption in the Qy-region. Figure 4b zooms in on the spectra in the very early stage of iron depletion. At day 0, we observe the typical PSI broad emission band centered at 723 nm, caused by trapping at the red pigments of the PSI antenna (26). The small broad band between 680 and 700 nm covers a very small signal from the PSI core pigments, PSII emission with its maximum at 687.5 nm (27) and emission from the phycobilisomes at 685 nm (28). The emission bands at 648 and 666 nm are also assigned to the phycobilisomes (28). After one day of iron depletion, there is a decrease in emission of both the phycobilisomes and PSI. The decrease of PSI emission is significant and the red emission maximum is observed to blue-shift to 722 nm. After two days of iron depletion, both phycobilisome and PSI emission decrease even further. The PSI red emission maximum is now at 721 nm. On this second day, an emission peak is observed to arise at 685 nm and assigned to IsiA (17). The shoulder around 690 nm of the emission spectrum after 2 days, is assigned to PSII. After 3 days without iron, the emission at 685.5 nm has increased strongly, whereas the PSI red emission has again significantly decreased. The recorded IsiA fluorescence will originate from free IsiA, since IsiA bound to PSI efficiently transfers energy (12,29), and the free IsiA is likely present as aggregates (1,6). We conclude that the formation of free IsiA, most probably in the form of aggregates, occurs already in the early stage of iron depletion, when PSI is still present in significant amounts. In Figure 4b, also the spectrum after 18 days of growth without iron is plotted, which is scaled to the emission recorded after 3 days in order to see the contribution of the IsiA vibrational band to the emission around 720 nm. It can thus be observed that after 5 days without iron, there is virtually no PSI left in the cells. Up to day 18, the amount of free IsiA in the cells is increasing (Figure 4a).

In Figure 5a the fluorescence spectra are shown of the cells after 3 days of iron depletion as a function of temperature. At 5 K, both IsiA and PSI emission are observed at respectively 685.5 and 721 nm. Upon raising the temperature, the IsiA fluorescence is observed to significantly decrease in the temperature range from 0 to 40 K. On the other hand, in this temperature range, the PSI emission hardly decreases. At 40 K the emission from PSII becomes visible as a shoulder at 693
nm. Upon raising the temperature from 40 K to 240 K, both IsiA and PSI emission are seen to decrease similarly. From 5 to 77 K the IsiA emission in the *Synechocystis* sp. PCC 6803 cells is seen to become largely quenched. This strong temperature dependence was noted before by Ihalainen and co-workers (1) and is stronger than recorded before for any other native photosynthetic complex. From these emission spectra, the relative fluorescence quantum yield can be determined for IsiA. Figure 5b displays the relative fluorescence quantum yield of IsiA, recorded between 675 and 700 nm, as a function of temperature for the first 18 days after iron depletion. Interestingly, the fluorescence decrease observed between 5 and 25 K is the same after 2 and 18 days of iron depletion. The steep decrease of fluorescence yield at low temperatures reflects the very strong quenching by IsiA. The difference in fluorescence yield at temperatures above 25 K between day 2 and 18 is explained by the relative contribution of the phycobilisomes to the emission at 685 nm (28) with respect to IsiA. This contribution is larger after 2 days with respect to 18 days of iron depletion, when there will be no more phycobilisomes present (3). Since phycobilisome emission will not have such a strong temperature dependence, it causes the observed larger fluorescence yields in the early stage of iron depletion with respect to that at later times (> day 7, Figure 5b). We conclude that the IsiA fluorescence is already significantly quenched after 2 days of iron deficiency. Moreover, since IsiA fluorescence is observed as of day 2, unconnected IsiA must be present from day 2 already. This conclusion is based on the absence of significant IsiA fluorescence for the PSI*IsiA* 18 isolated complexes (17) which evidences efficient energy transfer from IsiA into PSI. Quenching of the excitation energy is thus shown to occur in IsiA-aggregates even under physiological conditions when significant amounts of PSI are still present. In Figure 5c the relative fluorescence quantum yield is displayed for IsiA (687 nm) and PSI (720 nm) in cells depleted from iron for 10 days as a function of temperature. It is noted that these experiments were done on a different batch of iron depleted cyanobacteria than the measurement of Figure 3, 4 and 5a,b. Apparently, the time scale on which PSI is no longer present after iron depletion can vary, because it is hard to control differences in culture conditions, which play an important role in this ‘biological’ phenomenon. The emission at 720 nm has been corrected for the contribution of the IsiA vibrational band (Figure 4b). Interestingly, from this curve it is observed that the fluorescence yield from PSI increases upon increasing the temperature from 5 to 40 K. This effect is stronger in cells of the PSI -FJ and -L mutant (30,31) (data not shown). We explain these data by an increased possibility of energy transfer from IsiA to PSI upon raising the temperature, avoiding local
traps in IsiA that give rise to 685 nm fluorescence at 5 K (17), which is due to larger domains in which the excitations travel during their lifetime. From the temperature dependence of the IsiA fluorescence the enormous excitation energy quenching of this complex at higher temperatures is obvious.

Fig 3. 5 K absorption spectra of *Synechocystis* sp. PCC 6803 cells after 0-18 days of iron depletion. The spectra are normalized to the Chl a absorption in the Q_y-region.

Fig 4a) 5 K emission spectra of *Synechocystis* sp. PCC 6803 cells after 0-18 days of iron depletion. The spectra are normalized to the Chl a absorption in the Q_y-region, b) zooms in on the emission spectra of the first days after iron depletion and the emission spectrum of day 18 is scaled to see the contribution of the vibrational band of IsiA-aggregates at wavelengths around 720 nm.
**Fig 5a)** Temperature dependence of the emission of *Synechocystis* sp. PCC 6803 cells grown 3 days without iron, **b)** the relative fluorescence yield of IsiA as determined from the peak at 685.5 nm as a function of temperature for 0-18 days of iron depletion, **c)** relative fluorescence quantum yield of PSI at 720 nm compared to that of IsiA at 685.5 nm in cells grown 10 days without iron; the PSI yield has been corrected for the IsiA fluorescence tail at 720 nm.
Conclusion

Time-resolved fluorescence measurements on IsiA-aggregates isolated from *Synechocystis* sp. PCC 6803 as a function of temperature revealed the fluorescence lifetime at 5 K of IsiA-aggregates to be much smaller than observed for monomeric CP47 at 77 K. It is concluded that IsiA-aggregates function as excitation energy quenchers already at cryogenic temperatures.

Iron deficient *Synechocystis* sp. PCC 6803 cells were studied as a function of time by low temperature steady-state fluorescence spectroscopy. Unconnected IsiA-aggregates were shown to be present already in the early stages of iron depletion. These aggregates serve a photo-protective role, as was shown by the occurrence of very strong quenching of the 687 nm fluorescence when significant amounts of PSI and PSII are still present. For a comparison, the temperature dependence of the fluorescence emission yield of LHCII aggregates (32,33) shows a comparable trend to that recorded for IsiA, though the extent of the decrease from 5 K to 77 K is much stronger in IsiA. The spectral behavior upon aggregation is also very different among LHCII and IsiA. In LHCII aggregates and three-dimensional crystals there is a significant temperature-dependent red-shift of the fluorescence upon aggregation (32,34), whereas in IsiA there is only a very moderate red-shift upon aggregation. The red-shift in LHCII upon aggregation may be caused by increased pigment-pigment interactions, leading to a red-shift of absorption and emission and simultaneously a shortening of the fluorescence lifetime. The fact that the red-shift in IsiA is much less pronounced suggests that the quenching mechanism in both systems may be different.

We conclude that IsiA serves a dual role in cyanobacteria under iron deficient conditions: IsiA associates with PSI to enlarge the absorption cross-section of PSI (this study Fig. 5c, (11,12)) and at the same time unconnected IsiA-aggregates provide photoprotection for the whole organism (this paper, (1,35)), probably both with the purpose to control the redox state of the plastoquinone pool. More research is needed to reveal the mechanism by which these two processes are tuned.

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