Spectroscopic properties of PSI–IsiA supercomplexes from the cyanobacterium *Synechococcus* PCC 7942

Elena G. Andrizhiyevskaya a, Tatjana M.E. Schwabe b, Marta Germano a, Sandrine D’Haene a, Jochen Kruij b, Rienk van Grondelle a, Jan P. Dekker a, *

a Division of Physics and Astronomy, Institute of Molecular Biological Science, Faculty of Sciences, Vrije Universiteit, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands
b Lehrstuhl Biochemie der Pflanzen, Ruhr-Universität Bochum, D-44780 Bochum, Germany

Received 30 July 2002; received in revised form 2 October 2002; accepted 16 October 2002

Abstract

The cyanobacterium *Synechococcus* PCC 7942 grown under iron starvation assembles a supercomplex consisting of a trimeric Photosystem I (PSI) complex encircled by a ring of 18 CP43 or IsiA light-harvesting complexes [Nature 412 (2001) 745]. Here we present a spectroscopic characterization by temperature-dependent absorption and fluorescence spectroscopy, site-selective fluorescence spectroscopy at 5 K, and circular dichroism of isolated PSI–IsiA, PSI and IsiA complexes from this cyanobacterium grown under iron starvation. The results suggest that the IsiA ring increases the absorption cross-section of PSI by about 100%. Each IsiA subunit binds about 16–17 chlorophyll *a* (*Chl* *a*) molecules and serves as an efficient antenna for PSI. Each of the monomers of the trimeric PSI complex contains two red chlorophylls, which presumably give rise to one exciton-coupled dimer and at 5 K absorb and fluoresce at 703 and 713 nm, respectively. The spectral properties of these C-703 chlorophylls are not affected by the presence of the IsiA antenna ring. The spectroscopic properties of the purified IsiA complexes are similar to those of the related CP43 complex from plants, except that the characteristic narrow absorption band of CP43 at 682.5 nm is missing in IsiA.

© 2002 Elsevier Science B.V. All rights reserved.

Keywords: Photosystem I; PSI-IsiA; CP43; *Synechococcus* PCC 7942

1. Introduction

In cyanobacteria grown under normal conditions, light for photosynthesis is harvested by the intrinsic core antenna systems of Photosystem I (PSI) and Photosystem II (PSII) and by phycobilisomes, which are large supramolecular membrane-extrinsic peripheral light-harvesting complexes consisting of phycobilin proteins [1]. Under conditions of iron deficiency, however, the phycobilisomes are rapidly degraded and a chlorophyll *a* (*Chl* *a*) binding protein called IsiA or CP43/V is expressed in large quantities [2]. For the cyanobacteria *Synechocystis* PCC 6803 and *Synechococcus* PCC 7942, it has recently been shown that 18 molecules of IsiA can encircle a trimeric PSI complex [3–5], thus forming the so-called PSI–IsiA supercomplexes. The antenna ring around PSI probably increases its light-harvesting capacity under conditions when formation of other light-harvesting subunits is impossible. The analysis of light-saturation curves of PSI and PSI–IsiA from *Synechococcus* PCC 7942 revealed that the antenna ring around PSI increases the cross-section of light harvesting by 44% [4]. Complexes structurally similar to the PSI–IsiA supercomplexes were found in oceanic *Prochlorococcus* species, in which 18 membrane-intrinsic *Chl* *a*/*b* binding Pchb antenna proteins were shown to surround the trimeric PSI complex [6]. These species grow at a considerable depth with limited access of light and benefit from the additional Chl antenna in order to increase the light-harvesting capacity for PSI.

The efficiency of energy transfer from the IsiA antenna ring to the trimeric PSI core complex as well as the spectral arrangement of the chlorophylls in the PSI–IsiA supercomplex still have to be determined. From the analysis of 77 K emission spectra of isolated PSI–IsiA, PSI and IsiA complexes of *Synechocystis* PCC 6803, it was proposed [6]...
that the efficiency of energy transfer from the antenna ring to PSI is very high. Possible candidates for efficiently receiving excitation energy from the IsiA ring are a number of Chl molecules located at ideal positions near the outside rim of the PSI complex [7].

The spectral characteristics of IsiA have not yet been analyzed in detail. It is known that the appearance of a 685 nm emission peak at low temperatures serves as a marker for the presence of IsiA [2,8]. IsiA has a strong sequential homology to the CP43 core antenna protein of PSII, which has been studied in detail, both structurally [9] and spectroscopically [10–12]. The structure of CP43 was therefore used as a model for the organization of IsiA in the PSI–IsiA complex [3–5].

One of the typical features of almost all PSI complexes is the presence of so-called red pigments, which absorb at longer wavelengths than the primary electron donor P700. The amount and spectral characteristics of the red chlorophylls can differ significantly between different cyanobacterial species [13–15]. The characteristics of the red chlorophylls in the PSI complex from Synechococcus PCC 7942 are unknown.

In this work, we present a spectroscopic characterization of isolated PSI trimers, PSI–IsiA supercomplexes and IsiA complexes from Synechococcus PCC 7942 grown under iron starvation. From an analysis of temperature-dependent absorption and emission spectra obtained with nonselective or selective excitation, we conclude that the red chlorophylls in the PSI complex of Synechococcus PCC 7942 are spectrally different from those observed in other cyanobacteria, and that the addition of 18 IsiA complexes to the central trimeric PSI complex increases the light-harvesting capacity of PSI by a factor of 2.

### 2. Materials and methods

PSI–IsiA and PSI particles were isolated and prepared from the cyanobacterium Synechococcus PCC 7942 grown for 2 days in medium without added iron [4] using the methods described in Ref. [16]. In brief, thylakoid membranes were solubilized with 1.1% n-dodecyl-β-D-maltoside (β-DM) and passed over an anion exchange column, which allowed separation of PSI trimers and PSI–IsiA supercomplexes. A following hydrophobic interaction chromatography provided further purification. PSI–IsiA was further purified by size-exclusion chromatography (SEC), using two different columns (Superdex 200 and Superose 6, both HR 10/30, from Pharmacia) connected in series, in an FPLC system equipped with a Waters 990 diode array detector. The composition of the buffer used as mobile phase was 20 mM Bis–Tris, pH 6.5, 5 mM MgCl₂ and 0.03% β-DM. The SEC runs were performed at 25 ml h⁻¹, under which conditions PSI–IsiA had a retention time of 43 min. A small peak observed at 66 min was identified as practically pure IsiA by comparison of its absorption spectrum with those of PSI and of PSI–IsiA (not shown). The IsiA sample used in the spectroscopic measurements described here corresponds to what was collected from the 66 min peak of the SEC runs.

For spectroscopic measurements, all samples were diluted in a buffer containing 20 mM Bis–Tris (pH = 6.5), 10 mM MgCl₂, 10 mM CaCl₂ and 0.03% β-DM, which was supplemented with 66% (w/w) glycerol for low-temperature measurements. The OD of the samples used for the fluorescence and absorption measurements was about 0.1 and 0.6 cm⁻¹, respectively, at the Q₅ absorption maximum.

Room temperature absorption spectra were recorded on a Perkin Elmer Lambda 40 UV/VIS spectrometer. Temperature-dependent absorption measurements were recorded with a spectral resolution of 0.5 nm on a home-built spectrophotometer as described in Ref. [17]. Fluorescence excitation spectra at 5 K were recorded on a home-built fluorometer as described in Ref. [17], using an interference filter with transmission maximum at 730 nm and full width at half maximum (fwhm) of 10 nm to collect the fluorescence. The anisotropy of the emission, induced by vertically polarized excitation light, is defined as

\[ r = \frac{I_v - I_h}{I_v + 2I_h}, \]

where \( I_v \) and \( I_h \) are the fluorescence intensities with vertical and horizontal detection beam polarizations, respectively.

Fluorescence spectra were measured with a cooled CCD camera (Cromex ChromCam), equipped with a 1/2-m spectrogaph (Chromex 500IS). For nonselective fluorescence measurements, a 150-W tungsten halogen lamp in combination with an interference filter (with a transmission maximum at 420 nm and a fwhm of 20 nm) was used as excitation source. A cut-off filter was used to protect the CCD from scattered light. For site-selective fluorescence measurements, a cw dye laser (Coherent CR599) with DCM dye, pumped by an argon ion laser (Coherent Innova 310), was used. The excitation power was kept below 100 μW/cm² and the spectral resolution was 0.5 nm.

Circular dichroism spectra were recorded on a home-built spectrogaph described in Ref. [18].

### 3. Results

#### 3.1. Absorption

Fig. 1 shows the room temperature absorption spectra of the isolated PSI–IsiA, IsiA and PSI complexes of the cyanobacterium Synechococcus PCC 7942. In the Q₅ absorption region of the chlorophylls, the spectra peak at 674, 669 and 679 nm respectively. The absorption between 450 and 550 nm originates predominantly from β-carotene. This absorption is rather low in the free IsiA complexes, which can be explained by the removal of some β-carotene molecules during the preparation of the free IsiA complexes. Such molecules are vulnerable to detergent-induced removal from the complex if they are located near the contact regions of neighboring IsiA complexes or of IsiA and PSI.
Upon decreasing the temperature, the absorption spectra show more structure (Fig. 2A, B). The 5 K absorption spectrum of PSI–IsiA (Fig. 2A, solid line) shows two main absorption bands peaking at 679 and 668 nm. The PSI spectrum peaks at 679–680 nm at all temperatures between 5 and 293 K (Fig. 2B) and has an isosbestic point at 689 nm. A Gaussian decomposition of the 5 K absorption spectrum of the PSI complex (Fig. 2C) gives components with maxima around 662, 669, 679, 688, 697 and 703 nm. The 697 nm band has the oscillator strength of two chlorophylls and can be assigned to the primary electron donor, P700. The 703 nm band also has the oscillator strength of two chlorophylls and is primarily caused by the so-called red chlorophylls (see also below). The 703 nm band has smaller intensity and is less red-shifted than the absorption bands of the red chlorophylls in the cyanobacteria Synechocystis PCC 6803, Synechococcus elongatus and Spirulina platensis [14,19]. The absorption spectrum of the PSI–IsiA complex is similar to that of the PSI complex in the red part of the spectrum, implying that the absorption properties of the red chlorophylls are not affected by the association of the peripheral IsiA antenna. We note that the room temperature and 5 K absorption spectra of the PSI trimers were identical in trimers obtained from cells grown iron-deficient and iron-rich medium (not shown). This indicates that the spectral properties of the (red) chlorophylls are not affected by the presence of iron during growth.

In Fig. 3, the 5 K absorption spectra of the PSI–IsiA, PSI and IsiA complexes are plotted with normalized area under the curves. The data show that the IsiA complex dominates the absorption between 660 and 670 nm, and that the absorption at wavelengths longer than 698 nm originates exclusively from the PSI complex. The 5 K absorption spectrum of IsiA resembles that of CP43 [10,11], except that the extremely narrow absorption feature of CP43 at 682.5 nm is absent in IsiA.
The absorption spectrum of the PSI–IsiA complex cannot be exactly reconstituted from the absorption spectra of the individual IsiA and PSI complexes. The best fit was obtained with Q_y cross-section areas of 52% and 48% for IsiA and PSI, respectively (Fig. 3). Applying this proportion, a major part of the reconstituted spectrum matches the absorption spectrum of PSI–IsiA quite well, including the part (697–710 nm) where IsiA does not absorb at all. If we assume that each PSI monomer of *Synechococcus* PCC 7942 contains 96 chlorophyll molecules, as in PSI from *S. elongatus* [7], then the numbers of chlorophylls corresponding to that fit are 288 for the PSI trimer and 312 for the 18 IsiA units. This gives approximately 17–18 chlorophylls in each IsiA unit, which is a considerably larger number than found by X-ray crystallography for the CP43 protein of *S. elongatus* [9]. Note that the molecular models of the PSI–IsiA complexes presented by Bibby et al. [3,5] and Boekema et al. [4] assume 12 chlorophylls per IsiA protein.

### 3.2. Fluorescence emission upon nonselective excitation

Nonselectively excited (λ_ex = 420 nm) emission spectra of the PSI–IsiA, PSI and IsiA complexes are shown in Fig. 4. At room temperature (Fig. 4A), the emission bands of the purified IsiA and PSI complexes have maxima at 678 and 690 nm, respectively. The fluorescence spectrum of PSI–IsiA peaks at 686 nm and probably represents combined emission from the IsiA antenna and the PSI core complexes.

The fluorescence spectrum of IsiA at 5 K peaks at 682 nm and has an fwhm of 9.8 nm (Fig. 4B). The peak maximum occurs at about the same wavelength as that of the isolated CP43 complex from spinach [10]. However, the spectrum of CP43 has a much narrower fwhm of 4.5 nm. The 5 K emission spectrum of the PSI complex is dominated by a broad peak at 714 nm, but there are also small shoulders at 690 and 695 nm (Fig. 4B). Upon increasing the temperature, the main peak shifts to longer wavelengths (Fig. 5B), and at 77 K the maximum is at 718 nm. In PSI complexes from *Synechocystis* PCC 6803 [20] and *S. elongatus* [21], only a very small red shift of the emission maximum was observed upon increasing the temperature. At temperatures higher than 180 K, the emission from the bulk antenna dominates the fluorescence. The temperature-dependent emission spectra of PSI have an isosbestic point at 689 nm, the same wavelength as the isosbestic point of the temperature-dependent absorption spectra (Fig. 2B).

The 5 K emission spectrum of the PSI–IsiA complex is characterized by a major peak at 713 nm and a minor peak at 685 nm (Fig. 4B). It is highly unlikely that the 685 nm fluorescence arise from free or disconnected IsiA complexes, because (1) free IsiA complexes were biochemically removed just before measurements; see Materials and methods, (2) the 685 nm fluorescence is red-shifted compared to that in free IsiA (Fig. 4B), and (3) the 685 nm fluorescence in the supercomplex has a much stronger temperature dependence than that in free IsiA (Fig. 5C). The 713 nm peak has a similar temperature dependence (Fig. 5A) as the 714 nm peak in the PSI core complex (Fig. 5B). The maximum and the amplitude of the 685 nm peak depend significantly on the temperature. The emission at this wavelength probably originates from the IsiA antenna, because it is not observed in the 5 K emission spectrum of the PSI complex (Fig. 4B). However, the peak position is 3 nm (64 cm⁻¹) red-shifted compared to the emission maximum (682 nm) of the isolated IsiA complex. Energy transfer among the IsiA units and/or pigment–pigment interactions between the IsiA subunits or between the IsiA and PSI parts of the PSI–IsiA supercomplex may cause this red-shift of the 5 K emission maximum. Site-selected emission measurements provide further evidence that the 685 nm fluorescence originates from IsiA (see below).

Fig. 5C shows the temperature dependence of the fluorescence yield of the PSI–IsiA and PSI complexes. Both complexes show a more pronounced temperature dependence than PSI complexes from *Synechocystis* PCC 6803 [20] and *S. elongatus* [17]. The stronger temperature dependence is most likely caused by the shorter absorption wavelength of the red chlorophylls in *Synechococcus* PCC 7942, which facilitates uphill energy transfer from the red chlorophylls to the fluorescence quencher P700 at intermediate temperatures. The slightly lower fluorescence yield of the PSI–IsiA complex between 40 and 77 K (Fig. 5C, circles) can be explained by the relatively strong temper-

---

Fig. 4. Nonselectively excited fluorescence emission spectra of IsiA, PSI and PSI–IsiA at room temperature (A) and 5 K (B).
ature dependence of the 685 nm IsiA peak. Apparently, the thermal energy in this temperature range is sufficient to allow energy transfer from the IsiA antenna to $P_{700}$ and/or the red chlorophylls, thus avoiding the local traps in IsiA that give rise to the 685 nm fluorescence at 5 K.

3.3. Fluorescence emission upon selective excitation

The excitation spectrum of the anisotropy of the 5 K fluorescence around 730 nm of the PSI–IsiA complex is shown in Fig. 6. The data indicate that at excitation wavelengths shorter than 690 nm, the anisotropy is close to zero. Starting from 693 nm, the anisotropy increases to a value of about 0.14 at 705 nm. The rise of the anisotropy starts at about the same wavelength as in the PSI complex from *Synechocystis* PCC 6803 [20], which suggests that selective excitation of red chlorophylls starts at about the same wavelength in both organisms.

For a further analysis of the origin of the fluorescence bands and the efficiency of energy transfer within the PSI–IsiA supercomplex, we recorded 5 K emission of the PSI–IsiA and PSI complexes with selective laser excitation. Fig. 7A shows emission spectra excited in the range 650–690 nm, normalized at their 713 nm maxima. The amplitude of the 685 nm emission band (Fig. 7B, diamonds) is proportional to the difference in absorption between IsiA and PSI (Fig. 7B, solid line). Site-selective emission measurements on PSI in the same wavelength range did not reveal any changes in the shape of the emission spectra (data not shown). These results confirm that the 685 nm emission of the PSI–IsiA complex originates from the IsiA antenna.

Excitation at wavelengths longer than 690 nm reveals that the peak wavelength of the emission ($\lambda_{em}$) strongly depends on the excitation wavelength ($\lambda_{ex}$), both for PSI–IsiA and PSI (Fig. 8). With increasing $\lambda_{ex}$, $\lambda_{em}$ first shifts to shorter wavelengths by about 2.5 nm until, both in PSI–IsiA and in PSI, a minimal value is observed at $\lambda_{ex} \approx 698$ nm. A

---

**Fig. 5.** Temperature dependence of fluorescence emission of PSI–IsiA (A) and PSI (B). The spectra were normalized to the value corresponding to the maxima of the 5 K emission to compare the intensities of the fluorescence at different temperatures. (C) Relative fluorescence yield as a function of temperature, calculated by integrating the emission spectra at each temperature. The data were obtained by nonselective excitation at 420 ± 10 nm.

**Fig. 6.** Anisotropy of fluorescence (solid line) compared to the absorption spectrum of PSI–IsiA (dashed line) measured at 5 K. The dashed part of the anisotropy spectrum indicates the range where the anisotropy value was calculated with low accuracy.
further increase of $\lambda_{\text{ex}}$ causes $\lambda_{\text{em}}$ to shift to longer wavelengths. The original position of $\lambda_{\text{em}}$ (the zero-crossing point) is observed at $\lambda_{\text{ex}} = 703$ nm for both PSI and PSI–IsiA. A similar dependence of $\lambda_{\text{em}}$ on $\lambda_{\text{ex}}$ was observed for monomeric and trimeric PSI core complexes of *Synechocystis* PCC 6803 [20], except that the $\lambda_{\text{ex}}$ values giving rise to the minimum and zero-crossing $\lambda_{\text{em}}$ values are about 5 nm blue-shifted in the PSI and PSI–IsiA complexes of *Synechococcus* PCC 7942. The wavelength dependence of the emission maximum of the 5 K PSI fluorescence from *Synechocystis* PCC 6803 was explained by inhomogeneous broadening of the absorption band of the red chlorophylls, combined with the absence of energy transfer [20]. It was also argued that the value of $\lambda_{\text{ex}}$ for the zero-crossing point of $\lambda_{\text{em}}$ coincides with the absorption maximum of the red chlorophylls [20]. By analogy, these results confirm that the peak position of the red chlorophylls is near 703 nm in *Synechococcus* PCC 7942.

3.4. Circular dichroism

The 77 K CD spectra of the PSI–IsiA, PSI and IsiA complexes are shown in Fig. 9. Compared to the CD spectrum of the PSI complex, the CD spectrum of PSI–IsiA shows a red-shifted negative maximum at 686 nm and a blue-shifted positive maximum at 671 nm and an additional up- and down-going feature around 678 nm. The red- and blue-shifted maxima and the feature around 678 nm are also observed in the CD spectrum of IsiA. The feature around 678 nm in the spectrum of IsiA is, however, not sharp enough to explain the PSI–IsiA spectrum. We note that the spectral and CD properties of IsiA can be affected by the association of IsiA into the PSI–IsiA supercomplex. Taken together, the results indicate that the CD spectrum of the PSI–IsiA supercomplex contains contributions from both the PSI and the IsiA subunits of the supercomplex.

4. Discussion

4.1. PSI from *Synechococcus* PCC 7942

The results presented in this contribution indicate that the PSI complex from *Synechococcus* PCC 7942 occupies a
new and until now unique position among the various species of cyanobacteria regarding the properties of the red chlorophylls. In this complex, the red chlorophylls give rise to 5 K absorption and fluorescence maxima at 703 and 713–714 nm, respectively, while their oscillator strength corresponds to that of two chlorophyll molecules. In contrast, the spectroscopically well-characterized PSI complexes from the cyanobacteria *Synechocystis* PCC 6803, *S. elongatus* and *S. platensis* all contain absorption bands peaking at 708 nm at 5 K with oscillator strengths of at least four Chl molecules [14,19,21–24], while *S. elongatus* and *S. platensis* contain additional red chlorophylls that absorb and fluoresce at even longer wavelengths. On the other hand, the primitive cyanobacterium *Gloeobacter violaceus* does not contain red chlorophylls at all [25,26].

Based on the large Stokes’ shift between absorption and fluorescence and other spectroscopic measurements, it has been proposed that the red chlorophylls in cyanobacterial PSI core complexes arise from excitonically coupled dimers or larger aggregates of Chl a [20,22,27–30]. The Stokes’ shift in PSI from *Synechococcus* PCC 7942 is about 200 cm$^{-1}$ and is very similar to that observed in *Synechocystis* PCC 6803 [20]. Therefore, we conclude that the red chlorophylls in *Synechococcus* PCC 7942, which we designate C-703, also arise from excitonically coupled Chl a molecules. Because the oscillator strength is not more than that of two Chl a molecules (Fig. 2C), it is most likely that C-703 arises from a single Chl a dimer. The spectral properties of C-703 appear not to be affected by the presence of the peripheral IsiA antenna.

C-703 shows a more pronounced red shift of the fluorescence upon increasing the temperature than the red chlorophylls of PSI complexes from other organisms. In addition, the decrease of the fluorescence quantum yield upon raising the temperature is more pronounced for C-703 than for other red chlorophylls, probably because the uphill energy transfer from the long-wavelength pigments to the primary electron donor P700 is more efficient. At intermediate temperatures (e.g., between 40 and 100 K), the uphill energy transfer to P700 will already be efficient for the population of C-703 absorbing on the blue side of the inhomogeneous distribution, whereas the energy for the C-703 population on the red side of the distribution will not be sufficient for uphill energy transfer. These red molecules will still fluoresce, and because the blue fluorescence is quenched, the spectrum will shift to the red with a concomitant decrease of fluorescence quantum yield. For the C-708 molecules of *Synechocystis* PCC 6803, more thermal energy is required on average for the uphill energy transfer to P700. As a consequence, the red shift and decrease of quantum yield will start at higher temperatures and will be less pronounced in PSI from this species.

We note that a broad inhomogeneous distribution of C-703 is not only required to explain the temperature dependence of the maximum and yield of the nonselectively excited emission spectrum, but is also needed to explain the dependence of the emission maximum ($\lambda_{\text{em}}$) on the excitation wavelength ($\lambda_{\text{ex}}$) in the case of selective excitation (Fig. 8). The minimal value of $\lambda_{\text{em}}$ in the plot of Fig. 8 deviates more from the $\lambda_{\text{em}}$ value observed with nonselective excitation than in any other cyanobacterial PSI complex [20,21], which can be explained by the presence of only one red dimer in *Synechococcus* PCC 7942 and at least two in the PSI complexes of *Synechocystis* PCC 6803 and *S. elongatus*, since energy transfer between the red chlorophylls will diminish the selectivity of excitation.

### 4.2. IsiA from *Synechococcus* PCC 7942

The results on the isolated IsiA complex presented in this contribution must be regarded as preliminary because of limited sample availability. However, a first comparison between the spectroscopic properties of IsiA and the related CP43 complex from spinach is possible. It was argued before [10–12] that CP43 from spinach contains two different red-most absorption bands, which both peak near 682 nm. One of those bands has an extremely narrow band width and an oscillator strength of about one chlorophyll, and is probably not excitonically coupled to other chlorophylls. Its 13$^1$ C=O stretch mode is at 1658 cm$^{-1}$ [10], indicative of strong hydrogen bonding to the protein. This strong hydrogen bond may be the cause of the red shift of the absorption of this chlorophyll to 682 nm. The second 682 nm absorption band has a much broader band width, is composed of several excitonically interacting chlorophylls, determines the CD spectrum, and has a 13$^1$ C=O stretching mode at 1671 cm$^{-1}$. Both bands contribute about equally to the 5 K steady-state emission spectrum [10].

The results presented in this contribution indicate that the general shapes of the CD and absorption spectra of IsiA and CP43 are very similar, except that the narrow band is missing in IsiA. The 5 K emission spectrum of IsiA is much broader than that of CP43, which can be explained by the absence of the narrow 682 nm band in IsiA. We conclude that the broad 682 nm band has similar properties in both complexes and suggest that it may originate from similarly positioned chlorophylls. Thus, if the idea is correct that these chlorophylls in CP43 can be identified as the ones known as Chl 23, Chl 27 and Chl 33 located at the stromal side of the protein [12], then IsiA may also contain chlorophylls at these positions.

The comparison of low temperature absorption spectra of the isolated IsiA and PSI complexes with the spectrum of PSI–IsiA (Fig. 3) revealed that the absorption cross-sections of IsiA and PSI in the supercomplex are about 52% and 48%, respectively. This means that IsiA should bind about 17 Chl molecules, assuming 96 Chl molecules in each PSI monomer. The number converts to 16 if it is assumed that PSI from *Synechococcus* PCC 7942 binds only about 90 Chl molecules. This stoichiometry may be more realistic in view of the lower content of red chlorophylls in *Synechococcus* PCC 7942 than in *S. elongatus*. The crystal structure of
CP43 from *S. elongatus* has identified 13 Chl molecules [31], though a slightly larger number can probably not be excluded yet, since the resolution in that structure is limited. It is, however, also possible that IsiA contains more chlorophylls than CP43.

### 4.3. The PSI–IsiA supercomplex from *Synechococcus PCC 7942*

PSI–IsiA supercomplexes are assembled by cyanobacteria as a response to conditions leading to the disappearance of their main peripheral light-harvesting complexes, the phycobilisomes. Thus, one can expect that the main function of the IsiA ring around the trimeric PSI is to collect and deliver excitation energy to the reaction center of PSI.

The low-temperature emission spectrum of the PSI–IsiA complex shows a small peak at 685 nm (Fig. 7A), and site-selected emission measurements (Fig. 7) indicate that this peak originates from the IsiA complex. Its maximum is 3 nm (64 cm\(^{-1}\)) red-shifted compared to that of the isolated IsiA complex, and its fluorescence yield has a pronounced temperature dependence, contributing to about 12% of the total emission of the PSI–IsiA complex at 5 K. At slightly higher temperatures, the relative intensity of the 685 nm band decreases significantly, indicating very efficient energy transfer from IsiA to PSI at these temperatures. At room temperature, a significant part of the fluorescence arises again from the IsiA antenna, but in this case, the excitation energy is probably completely delocalized over all chlorophylls before trapping by charge separation occurs. A more detailed picture of the processes of energy migration in the PSI–IsiA supercomplexes can be obtained from time-resolved fluorescence and/or transient absorption measurements.

### Acknowledgements

This work was supported by the Netherlands Foundation for Scientific Research (NWO) via the Foundation for Life and Earth Sciences (ALW). Financial support by the Fonds der chemischen Industrie (J.K) and the Deutsche Forschungsgemeinschaft (SFB 480, C2-JK) is gratefully acknowledged. Elfriede Pistorius and her group deserve special thanks for providing cell material of *Synechococcus PCC 7942* and we (J.K., T.M.E.S.) thank Matthias Rögnér for his continuous support of our work.

### References


