Changes in the LHCI aggregation state during iron repletion in the unicellular red alga *Rhodella violacea*

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Abstract **Red algae are well suited to study the effects of iron deficiency on light-harvesting complex for photosystem I (LHCI), since they are totally devoid of light-harvesting complex for photosystem II (LHCII). Iron starvation results in a reduction of the pigment content, an increase of the fluorescence yield and a new emission band at 705 nm in the 77 K fluorescence emission spectra. These changes reflect the accumulation of uncoupled, aggregated LHCI in iron-depleted cells. Reconnection of LHCI to de novo synthesized reaction center I (RCI) is the first event, which takes place after iron addition. The changes in the aggregation state of LHCI are likely to occur also in brown and green algae.**

Key words: Fluorescence; Iron deficiency; Light-harvesting complex; Iron repletion; Photosystem I assembly; *Rhodella violacea*

1. Introduction

The photosynthetic apparatus is rich in iron-containing cofactors and pigment biosynthesis is regulated by iron. Iron limitation has therefore pleiotropic effects on photosynthesis in plants, algae and cyanobacteria [1–4]. Three effects of iron limitation are common to all species: a drastic reduction in the pigment content, an increased fluorescence yield and modifications in the 77 K fluorescence emission spectra [4–8]. In cyanobacteria, the increased fluorescence yield is due a chlorophyll–protein complex (CP43′) synthesized during iron depletion. It exists in two forms: one free in the membrane and fluorescing at 685 nm [9] and the other forming a ring around photosystem I (PSI) trimers as an antenna [10]. In green algae, there is an increase in the 77 K fluorescence emission at 710 nm attributed to modifications in the organization and efficiency of photosystem II (PSII) and its light-harvesting complex (LHCII) [4,7]. A disconnection of part of the PSII antenna caused by iron deficiency has also been observed in higher plants [11]. In red algae, light for PSII is collected, like in cyanobacteria, by a phycobilisome (PBS) [12,13]. Light for PSI is collected by the eukaryotic transmembrane light-harvesting complex I (LHCI), which binds chlorophyll *a* (Chl *a*) and zeaxanthine (Zea) [14–18]. Red algae devoid of LHCII are ideal to specifically study the effects of iron deficiency on LHCI.

In iron-starved cells of the unicellular red alga *Rhodella violacea*, the number of thylakoids is decreased with a 90% loss of the Chl content, while the content of PBP is only halved. LHCI polypeptides are in excess compared to PSI core complexes. The 77 K fluorescence emission spectra are modified with a shift of the maximum from 716 to 709 nm [8].

In this paper, we specifically studied the changes in the pigment content and fluorescence emission spectra occurring during iron repletion of cells of *R. violacea* grown for 4 weeks with a limited supply of iron.

2. Materials and methods

2.1. Cell culture

R. violacea (strain 115-79 from Göttingen University) was grown photoautotrophically in sterile artificial sea water (ASW, [19]) containing 0.125 μmol l^{-1} of $FeCl_3$ (Fe/80 medium) with the addition of vitamin B12 (25 μg l^{-1}). Cultures of 400 ml were incubated at 20°C in glass flasks continuously flushed with sterile air, and illuminated at 80 μE m^{-2} s^{-1} with white fluorescent cooled tubes in a 16 h light/8 h dark regime. The cells were diluted every week to 500 cells mm^{-3} in fresh Fe/80 medium for 4 consecutive weeks. On the first day of the 5th week, cells were diluted to 500 cells mm^{-3} either in the same medium for the control, or in the medium supplemented with 10 μmol l^{-1} of $FeCl_3$ to study iron repletion. Cells were counted on a Thoma cell with the NIH image public domain software. For translation inhibition, the cultures were diluted to 500 cells mm^{-3} on their 5th week of iron deficiency and chloramphenicol or cycloheximide were added to a final concentration of 200 μg ml^{-1} and 2 μg ml^{-1} respectively to inhibit expression of chloroplastic or nuclear genes respectively [20]. After 20 min incubation, 10 μmol l^{-1} of $FeCl_3$ was added to the culture.

2.2. Pigment analysis

PBS and Chl were extracted and the concentrations measured as described in [12,21]. Optical densities were measured with a spectrophotometer (Varian DMS, Sunnyvale, CA, USA). Pigment analyses were performed by high-performance liquid chromatography (HPLC) as previously described [22].

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Abbreviations: β-car, β-carotene; Chl, chlorophyll; LHCI and LHCII, light-harvesting complexes for photosystems I and II; P_{700}, photosystem I activity; PBS, phycobilisome; PSI and PSII, photosystems I and II; RCI, reaction center I; Zea, zeaxanthine

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2.3. 77 K fluorescence emission spectra

The 77 K fluorescence emission spectra were recorded on a Hitachi F-3010 Fluorescence Spectrophotometer. The excitation was set to 440 or 550 nm. Emission was scanned from 570 to 800 nm. Cell suspensions were dark-adapted for 15 min prior to any recording. A volume corresponding to 1 μg Chl was filtered and the filter immediately plunged into liquid nitrogen. For quantitative comparison of the 77 K emission spectra, rhodamine-B deposited on the filter prior to freezing was used as a standard. The difference spectra and their second derivatives were calculated using Gramms 32 software from the Galactic Industrial Corporation, USA.

2.4. Room temperature fluorescence induction

F_o and F_m levels were measured using a pulse amplitude-modulated (PAM) fluorimeter (model 101, Heinz-Walz, Effelrich, Germany) at 20°C as previously described [23].

2.5. Photosystem I activity (P_{700}) quantification

P_{700} was quantified by measuring the dark-minus-light absorbance at 700 nm according to [24].

3. Results

To obtain iron-depleted cells with a sustained growth we grew *R. violacea* in the presence of a limited amount of iron. All the phenomena described in [8] occurred to a lesser extent (see Section 1). After 4 weeks of culture, the medium was supplemented with iron and the fast recovery process was studied.

3.1. Pigment synthesis, and reaction center I (RCI) activity during iron repletion

When iron was added to the iron-limited cells, the pigment content and the PSI activity rapidly increased. The larger variations were those of Chl and β-carotene (β-car), the increase in Zea and PBS being weaker (Table 1). Zea is bound to LHCI while β-car is in the core complex of photosystems [16,18,25]. Therefore the synthesis of core complexes was the largest. The RCI de novo synthesis was reflected in the increase of the P_{700} activity. When compared to the increase in Zea concentration, it became obvious that the relative amount of RCI per LHCI was increasing. If arbitrarily taken equal to 1 in repleted cells, it was lowered to 0.17 in iron-depleted cells (Table 1).

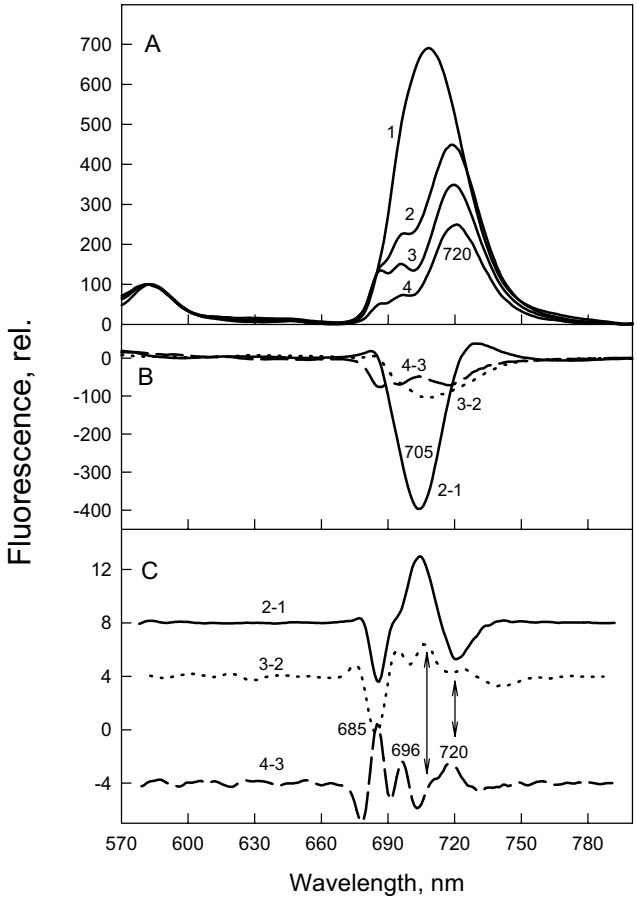

Fig. 1. A: 77 K fluorescence spectra of *R. violacea* cells measured before (1) and during iron repletion: 32 h (2), 48 h (3), and 72 h (4) (excitation at 440 nm). 1 μg of Chl *a* and a constant quantity of rhodamine B fluorescing at 586 nm were deposited on filters before freezing. The spectra were normalized to the peak at 586 nm. B: Corresponding difference spectra 2-1, 3-2 and 4-3. C: Second derivative spectra of the difference spectra from B.

4 days after iron supplementation, the Chl/P_{700} ratio was similar to that obtained with PSI fractions from unstressed algae [8,15,17].

Table 1
Time after iron supplementation

Line no.	Pigment concentrations and P_{700} concentration	Time after iron supplementation (days)				
0		0	1	2	3	4
1	Chl (fmol/cell)	0.61	0.88	1.75	3.78	4.55
	Chl/Chl initial		*1.43*	*2.85*	*6.14*	*7.4*
2	Zea (fmol/cell)	0.35	0.42	0.52	0.91	1.00
	Zea/Zea initial		*1.16*	*1.48*	*2.60*	*2.85*
3	β-car (fmol/cell)	0.21	0.30	0.45	0.79	0.95
	β-car/β-car initial		*1.50*	*2.14*	*3.76*	*4.52*
4	PBS pg/cell	13.07	15.65	21.83	27.04	27.20
	PBS/PBS initial		*1.19*	*1.6*	*2.06*	*2.08*
5	Chl/P_{700} (mol/mol)	554	314	240	288	249
6	Zea/P_{700} (mol/mol)	317	150	71	69	55
7	Relative P_{700}/LHCI	0.17	0.37	0.77	0.80	1

In line 0, the time after iron supplementation is indicated in days, the cellular content in fmol of Chl is indicated in line 1, the ratio of Chl to the initial Chl is indicated in italics. Lines 2 and 3 correspond to the HPLC determination of the Zea and β-car concentration in the cell, the ratio of their concentration to the initial concentration is indicated in italic. Line 4 indicates the spectrophotometric determination of PBS after its purification, the ratio of PBS to the initial PBS is indicated in italic. Line 5 gives the ratio Chl/P_{700} (mol/mol). Line 6 is deduced from lines 1, 2, and 5. For line 7, the inverse of line 6 values were normalized to the value found after 4 days of iron supplementation.

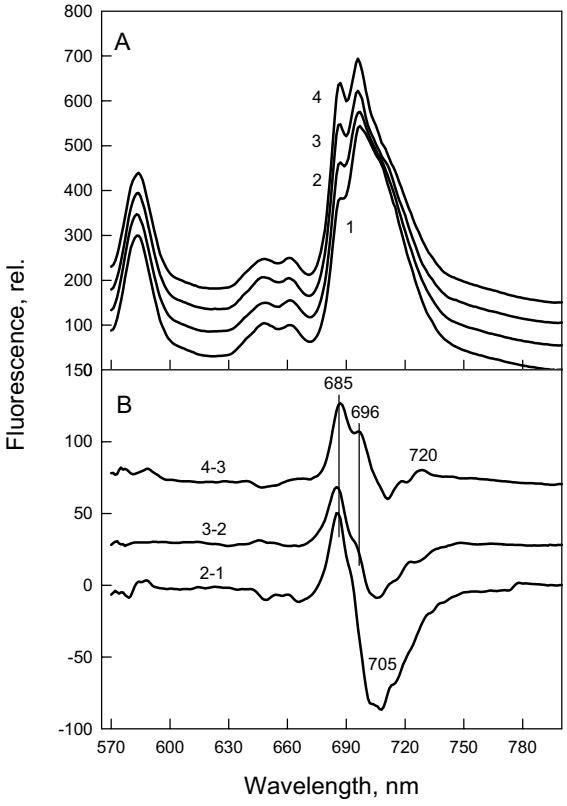

Fig. 2. A: 77 K fluorescence emission spectra of *R. violacea* cells (excitation at 550 nm) during iron repletion. 1: 0 h, 2: 3 h, 3: 6 h, 4: 9 h. The spectra are normalized to the peak at 662 nm (corresponding to the allophycocyanin). B: Corresponding difference spectra: 2-1, 3-2 and 4-3.

3.2. Fluorescence changes during iron repletion

Modifications in the 77 K fluorescence emission spectra (Figs. 1 and 2) and in the room temperature fluorescence (Fig. 4) occurred during repletion. At 77 K, with the excitation at 440 nm, it was obvious that on a Chl basis, the cells grown under a limited supply of iron emitted more fluorescence (spectrum 1, Fig. 1A). The emission spectrum of iron-depleted cells was dominated by a broad band with a peak at 705 nm. During iron repletion, the amplitude decreased and the peak of the maximum was shifted from 705 to 720 nm (spectra 1–4, Fig. 1A). Towards the end of the recovery process, the shoulders at 685 and 695 nm (attributed to PSII) and the peak at 720 nm (attributed to PSI), masked in depleted cells, became visible again. The main change from 0 (spectrum 1) to 32 h (spectrum 2) of iron repletion was a large decrease of the band at 705 nm, two other bands (720, 685 nm) were also visible. The changes occurring from 32 h of repletion to 72 h were less drastic. During the first hours of repletion, the emission spectra were also recorded with an excitation at 550 nm in the phycoerythrin absorption band. The spectra were arbitrarily normalized to 662 nm (Fig. 2A). The bands of phycoerythrin (585 nm), phycocyanin (650 nm) and allophycocyanin (662 nm) were always visible. In depleted cells, the maximum was shifted to 696 nm with shoulders at 685 nm and around 710 nm (Fig. 2A). After 9 h of repletion, peaks at 685, 695 nm have been increased and the peak at 705 nm has been decreased (Fig. 2B).

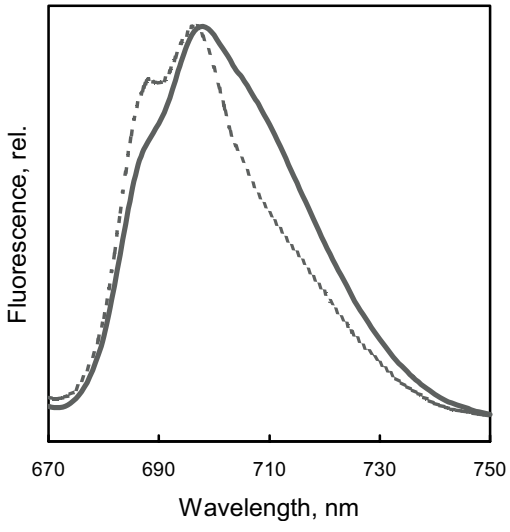

Fig. 3. 77 K fluorescence emission spectra of *R. violacea* cells during iron repletion in the presence of cycloheximide at concentration of 2 μg ml^{-1} (excitation at 550 nm). Time 0: solid line, 8 h: dotted line.

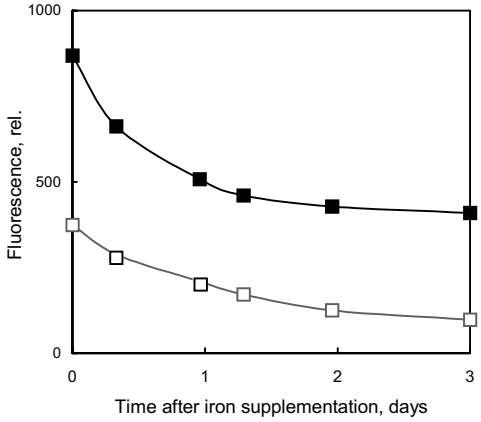

Fig. 4. Room temperature F_o (open squares) and F_m (solid squares) fluorescence levels of *R. violacea* cells at different times of iron repletion.

If repletion was done in the presence of chloramphenicol, which blocks the synthesis of the core complexes (encoded by the chloroplastic genome), the changes in the spectra were stopped. If repletion was done in the presence of cycloheximide, which blocks the synthesis of LHCI (encoded by the nuclear genome), changes still occurred during the first hours of iron repletion (Fig. 3).

At room temperature, the F_o and F_m levels were increased in depleted cells. During repletion they have been changed back to levels similar to those found in control cells (Fig. 4).

4. Discussion

In iron-depleted cells of *R. violacea*, the LHCI to RCI ratio is increased. LHCI in excess is unable to transfer the absorbed energy to RCI and will therefore contribute to the fluorescence emission at room temperature and at 77 K. At room temperature, in the control, PSI contributes moderately to the fluorescence emission [30]. However in iron-depleted cells, the rise of both the initial (F_o) and maximal fluorescence (F_m) levels suggests that uncoupled LHCI contributes to the fluorescence. The variable fluorescence $F_v = F_m - F_o$ is not likely to

be influenced by the participation of the LHCI to the fluorescence emission at room temperature and its increased value in depleted cells is an indication of the increased PSII to Chl ratio. At 77 K, our results show that the shift of the maximum from 720 to 705 nm is due to the large contribution of a band at 705 nm, suggesting that uncoupled LHCI fluoresces at 705 nm. As repletion begins, several modifications are visible: a diminution of the maximum fluorescence intensity, a shift of the maximum and changes in shape of the spectra. If we correlate the fluorescence data with the fast de novo synthesis of RCI and the increase of the ratio of active PSI centers relative to LHCI, we can propose that the decrease of the 705 nm band during the first hours corresponds to the binding of the pre-existing LHCI to the de novo synthesized RCI. This is further supported by the different responses to the translation inhibitors. The restored energy transfer between the pre-existing LHCI and the new RCI allows the decrease and the shift of the fluorescence emission. Minor changes are also occurring at different wavelengths, indicative of changes in energy transfer between antenna and core complexes.

The fluorescence spectrum of iron-depleted *R. violacea* resembles that of a RCI-less mutant of the green alga *Chlamydomonas reinhardtii*, where a fluorescence emission at 707 nm was attributed to aggregated LHCI polypeptides [26,27]. In vitro, monomeric LHCI from *Porphyridium cruentum* fluoresces at 677 nm [28], while dimers of LHCI from higher plants fluoresce at 702 and 730 nm [29]. We assume that, in vivo, in iron-depleted *R. violacea* cells, the uncoupled LHCI subunits are mostly organized in dimers giving the fluorescence peak at 705 nm. The number of LHCIs bound to the remaining RCIs in depleted cells might also be larger (increased antenna size).

The comparison with the case of cyanobacteria is interesting despite the total absence of LHC in these organisms. In cyanobacteria, the increased fluorescence yield and the shift of the maximum from 720 to 685 nm is due to CP43′ synthesized in excess during iron starvation, which completely disappears upon repletion. Some authors believe that CP43′ serves to store Chls which will be used for de novo synthesized PSI during iron repletion [31]. Despite its homology with CP43, which belongs to PSII, some CP43′ play the role of a PSI antenna forming a ring around RCI trimers. Our results suggest that in red algae, LHCIs in excess and free in the membrane (the equivalent of CP43′ in excess) do not disappear upon repletion but are used as pre-assembled antenna for re-synthesized RCI. Our interpretations of the events occurring in red algae differ from the attribution of very similar fluorescence changes in iron-depleted cells of a green alga to disconnected LHCII [7]. In iron-starved cells, LHCs can be detached from both photosystems and can contribute to the fluorescence changes. However, the 707 nm band of the RCI-less mutant of the green alga *C. reinhardtii* [26,27] is an evidence in favor of the participation of decoupled LHCI to the new fluorescence emission. The results obtained in red algae should be taken into account to interpret the effects of iron deficiency in other algae.

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