Effects of Carotenoid Inhibition on the Photosynthetic RC–LH1 Complex in Purple Sulphur Bacterium Thiorhodospira sibirica

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Effects of carotenoid inhibition on the photosynthetic RC–LH1 complex in purple sulphur bacterium *Thiorhodospira sibirica*

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Abstract

Core complexes (LH1–RC) were isolated using preparative gel electrophoresis from photosynthetic membranes of the purple bacterium, *Thiorhodospira sibirica*, grown in the absence or presence of the carotenoid biosynthesis inhibitor, diphenylamine. The biosynthesis of carotenoids is affected by diphenylamine both quantitavely and qualitatively: after inhibition, the level of carotenoids in core complexes reaches only 10% of the normal content, as analyzed by HPLC and absorption spectroscopy. The normally grown bacterium biosynthesizes spirilloxanthin, rhodopin, anhydrorhodovibrin and lycopene, whereas after inhibition only neurosporene, ζ-carotene and their derivatives are found in the complexes. There is no concomitant accumulation of appreciable amounts of colorless carotenoid precursors. Interestingly, the main absorption band of the core light harvesting complex isolated from carotenoid-inhibited cells, shows a red shift to 889 nm, instead of a blue shift observed in many carotenoid-deficient species of purple photosynthetic bacteria. The stability of isolated core complexes against *n*-octyl-β-D-glucopyranoside clearly depends on the presence of carotenoids. Subcomplexes resulting from the detergent treatment, were characterized by non-denaturating gel electrophoresis combined with *in situ* absorption spectroscopy. Core complexes with the native carotenoid complement dissociate into three subcomplexes: (a) LH1 complexes partially depleted of carotenoids, with an unusual spectrum in the NIR region (λ_{max} = 791, 818, 847 and 875 nm), (b) reaction centers associated with fragments of LH1, (c) small amounts of a carotenoidless B820 subcomplex. The core complex from the carotenoid-deficient bacterium is much less stable and yields only the two sub-complexes (b) and (c). We conclude that carotenoids contribute critically to stability and interactions of the core complexes with detergents.

Abbreviations: BChl – bacteriochlorophyll; Crt – carotenoid; DM – *n*-dodecyl-β-D-maltoside; DPA – diphenylamine; DPA-complex – complex isolated from the DPA-grown cell; *Chr.* – *chromatium*; *Ect.* – *Ectothiorhodospira*; HPLC – high performance liquid chromatography; LDS – lithium dodecyl sulfate; LH – light harvesting; $LH1_{fr}$ – fragment of LH1 complex; NIR – near infrared; β-*OG* – *n*-octyl-β-D-glucopyranoside; PAGE – polyacrylamide gel electrophoresis; *Rba.* – *Rhodobacter*; RC – reaction center; *Rsp.* – *Rhodospirillum*; *Trs.* – *Thiorhodospira*

Introduction

The photosynthetic apparatus of purple bacteria consists of reaction centers (RC) and a system of light harvesting (LH) antennas. The LH1 complex, also termed B880 according to its absorption maximum in the NIR, closely interacts with the RC and forms with it the so called

core complex B880-RC (or LH1-RC). Often, purple bacteria biosynthesize also auxiliary LH complexes, such as LH2, alternatively termed B800-850, which are located more peripherally (Drews 1985; Zuber and Cogdell 1995; Robert et al. 2003). The function of LH complexes is to efficiently absorb photons and transfer their energy as electronic excitation to the RC (van Grondelle et al. 1994).

Both types of antennae are assembled from very similar components: bacteriochlorophylls (BChls) and carotenoids (Crts) non-covalently bound to small hydrophobic polypeptides (molecular weight 6–8 kDa) named α and β, which are present in the native complexes in a 1:1 ratio. High-resolution X-ray crystallographic studies on LH2 from two species of purple bacteria revealed rings of 8 (or 9) αβ heterodimers, hosting two distinct groups of BChl: 8 (9) monomeric molecules, absorbing at 800 nm, and 16 (18) coupled BChls, with the near infrared (NIR) absorption maximum at 850 nm (McDermott et al. 1995; Karrasch et al. 1995; Koepke et al. 1996). Each αβ heterodimer also binds a single Crt molecule, which spans the depth of the complex. The structural analysis of carotenoidless LH1 antenna showed, with 8.5 Å resolution, a ring-shaped complex consisting of 16 αβ heterodimers, hosting 32 closely spaced BChl molecules (Karrasch et al. 1995). More recently, different sizes and considerable ring distortions in LH1 have been observed (Fotiadis et al. 2004) while a high-resolution structure of the core complex from *Rhodopseudomonas palustris* shows a gapped elliptical LH1 nearly surrounding the RC (Roszak et al. 2003). However, it appears that the latter structure may not apply to other species, and can also vary with growth conditions (Gerken et al. 2003). In a reconstituted LH1 complex from *Rhodobacter* (*Rba*). *sphaeroides*, a somewhat lower number of 20 ± 1 BChl *a* molecules forming the B875 ring has been estimated, using the excitation trap ([Ni]-BChl *a*) approach (Fiedor et al. 2000, 2001).

The polypeptides and BChl in LH complexes are indispensable for their functionally correct assembly and the BChls B880 (LH1) and B800 (LH2) can be manipulated within a limited range (Davis et al. 1996; 1997; Bandilla et al. 1998; Fiedor et al. 2001). Carotenoids are involved in photoprotection (Cogdell and Frank 1987; Frank and Cogdell 1996; Fiedor et al. 2002), and complement BChls in light harvesting (Ritz et al. 2000). They also play a structural role in LH complexes (Hladik et al. 1982; Jirsakova and Reiss-Husson 1994; Davis et al. 1995; Zurdo et al. 1995; Reinsberg et al. 2001; Fiedor et al. 2004), however, their content and type can vary more widely, and even be reduced to zero. Several methods can be used for the purpose of reducing or modifying the Crt content: (i) inactivation of genes for Crt biosynthesis; (ii) selective extraction; (iii) reconstitution with a desired pigment and (iv) biosynthesis inhibition.

Chemically-induced random mutagenesis resulted in carotenoidless (blue) mutants of purple bacteria, such as the G9 strain of *Rhodospirillum* (*Rsp*.) *rubrum* or R26 and R26.1 strains of *Rba. sphaeroides* (Griffiths and Stainer 1956; Sistrom et al. 1956). They lack colored carotenoids, but instead may accumulate their colorless precursors (Griffiths et al. 1955) and maintain the LH1 complex, albeit with a blue shifted NIR absorption. The LH2 complex is either absent (*Rba. sphaeroides* R26) or looses the binding site of BChl absorbing at 800 nm (strain R26.1) (Davidson and Cogdell 1981). More selective molecular genetic methods permit the interruption of Crt biosynthesis at various steps (Giuliano et al. 1988; Lang et al. 1995), or the insertion heterologous genes yielding non-native carotenoids (Garcia-Asua et al. 2002).

Selective carotenoid extraction has been used as an alternative route to carotenoid-less LH1 complexes which retain the original amino acid sequence of their polypeptides. Similarly to LH1 from the Crt-less mutants, they can be reversibly dissociated to Crt-less B820 subcomplexes (Chang et al. 1990; Heller and Loach 1990; Meckenstock et al. 1992; Moskalenko et al. 1992). The reversible dissociation is a very advantageous feature in reconstitution studies (Loach and Parkes-Loach 1995), which can be exploited to introduce non-native Crts into LH1 (Fiedor et al. 2004).

Another approach uses inhibitors to manipulate carotenoid biosynthesis (Goodwin and Osman 1953; Bramley 1993). This method has certain advantages in strains that cannot be transformed, and also allows a quantitative control. Crt-less photosynthetic membranes with low levels of precursors have e.g. been isolated from *Chromatium (Chr.) minutissimum*, *Ectothiorhodospira (Ect.)*

haloalkalopila and *Ect. mongolicum* M9 (Moskalenko et al. 1991).

Thiorhodospira (*Trs.*) *sibirica*, is a newly characterized species of purple photosynthetic sulphur bacterium from lake Malyi Kasutyi (Chita region, Russia) (Bryantseva et al. 1999). As spirilloxanthin is the main carotenoid in its cells it can be assigned to the group of bacteria with normal spirilloxanthin biosynthesis pathway (Takaichi 1999). The photosynthetic membranes isolated from this species show a complex NIR absorption, with four bands at 795, 830, 852 and 892 nm. According to a preliminary analysis, this is in part due to an unusual LH2 complex with three BChl absorption bands in the NIR region, which will be dealt with separately. We here report the isolation, fractionation and pigment analyses of core complexes from normally grown *Trs. sibirica* and from cells grown in the presence of diphenylamine as the inhibitor of carotenoid biosynthesis, and their dissociation in the presence of *n*-octyl-β-D-glucopyranoside (β-OG).

Materials and methods

Trs. sibirica (strain $A12^T$), isolated from soda lake Malyi Kasytui (the Chita region, Russia), was cultivated using the following medium (Bryantseva et al. 1999): 0.5 g KH_2PO_4; 0.5 g NH_4Cl; 0.5 g NaCl; 0.2 g $MgCl_2{\bullet}6H_20$; 0.05 g $CaCl_2{\bullet}2H_20$; 2.5 g $NaHCO_3$; 2.5 g Na_2CO_3; 0.5 g sodium acetate; 0.5 g sodium malate; 0.1 g yeast extract; 0.7 g $Na_2S{\bullet}9H_2O$ and 1 ml trace element solution SL8 per 1 l of distilled water; the pH was adjusted to 9.0–9.5. The bacterium was grown phototrophically at 25 °C under white light (20,000 lx). For the inhibition of carotenoid biosynthesis, diphenylamine (DPA) was added to the medium (12 mg/l), and the bacteria were grown under otherwise identical conditions as above. Light quality does not influence the inhibition process.

Chromatophores were isolated from cells disrupted by sonication, following the method previously described (Moskalenko and Erokhin 1974). The chromatophores were solubilized with dodecyl-maltoside (DM) and the core complexes (RC-LH1) were isolated by preparative polyacrylamide gel electrophoresis (PAGE) (Moskalenko and Erokhin 1974). The purified core complexes were further dissociated by incubation with 20% β-OG at room temperature under dim light. The resulting mixture was centrifuged (5000 *g*, 5 min) to remove insoluble material, and then separated by PAGE, performed in the presence of 0.8% β-OG in the gel (Moskalenko et al. 1997). Alternatively, the resulting complexes were separated by ion exchange chromatography on DEAE-Sepharose (CL-6B Fast Flow, Pharmacia), as described previously (Fiedor et al. 2001).

Polypeptide analysis by electrophoresis with lithium dodecylsulfate (LiDS-PAGE) was performed as published (Moskalenko et al. 1992). The photochemical activity of the RC fraction was measured with a home built phosphoroscope (Moskalenko and Kuznetsova 1993).

The HPLC equipment used for pigment analysis comprised a Shimadzu LC 10AD*vp* pump with a FCV 10Al*vp* low pressure gradient mixer, and two detectors: a Gilson 111B for absorption recording at 280 nm, and a Shimadzu UV160 spectrophotometer with HPLC flow cell for recording at a wavelength variable in 380–600 nm region. They were connected to two channel integrators (2000 Merck-Hitachi).

Pigments were extracted with an acetone–methanol mixture (7:2), transferred to petroleum ether, and dried in a stream of nitrogen. They were then dissolved in ethyl acetate or methylene chloride and separated without delay on a Separon C18 (Tessek, Czech Republic) reversed phase column (15×0.3 cm). The column was equilibrated in a solvent containing 27% ethyl acetate (A) and 73% (v/v) of 9:1 mixture of acetonitrile and water (B), and a linear gradient (from 27 to 100% A within 20 min, at flow rate of 0.6 ml/min) was started after injection. The entire procedure from pigment extraction to completion of the HPLC analysis took only 40 min. Fractions were collected automatically (Gilson 201 fraction collector), and their absorption spectra recorded on a Shimadzu UV-160 spectrophotometer. Carotenoids were identified by comparison with authentic samples obtained from various strains of bacteria, according to their absorption spectra and retention times. The amounts of each pigment were calculated using known extinction coefficients (Davies and Köst 1988; Britton et al. 2004). Recorded spectra were scanned and digitized using Graf2Digit program.

Results and discussion

Effects of inhibition

The presence of DPA in the growing medium of *Trs. sibirica* causes an almost complete inhibition of carotenoid biosynthesis in this organism. As estimated by absorption spectroscopy (Figure 1) and by HPLC, the content of Crts in photosynthetic membranes from inhibited cells drops below 10% of the normal content, and also changes qualitatively (Table 1). In non-inhibited cells, the main Crts are spirilloxanthin, rhodopin, anhydrorhodovibrin and lycopene. After inhibition only neurosporene, ζ-carotene and small amounts of their hydroxylated derivatives were identified. Interestingly, no phytoene was detectable after inhibition, which contrasts numerous works reporting on the accumulation of this precursor under the inhibitory action of DPA (Schmidt 1978). However, recently also in *Chr. minutissimum*, *Lamprobacter modestohalophillus* (Moskalenko et al. 1991) and in *Ect. haloalkalophila* (Moskalenko et al., unpublished) no accumulation of phytoene has been seen after DPA treatment. DPA has either other target(s) for inhibition of carotenoid biogenesis in these bacteria, besides the generally accepted phytoene desaturase (Schmidt 1978), or there are other regulatory mechanisms operating.

Core complexes were isolated by preparative non-denaturing PAGE, in the presence of the mild detergent, DM. A similar method was previously found effective in purification of core complexes from other purple bacteria (Moskalenko and Erokhin 1974; Moskalenko et al. 1997). The best yields of photosynthetic complexes from *Trs. sibirica* were achieved when running the gels in 0.8% DM. The spectrum of the core complex from uninhibited control cells (Figure 1, upper trace) has the main absorption maximum in the NIR at 883 nm (mainly LH1), and a minor one at 800 nm ('accessory' BChl in the RC), a shoulder around 760 nm (bacteriopheophytin in the RC), and maxima at 590 nm and 376 nm: assigned to the Q_x and Soret bands, respectively, of BChl in RC and LH1. The carotenoids give rise to a broad band, peaking at 508 nm, between the Q_x and Soret bands. The absorption spectrum of the core-complex from DPA-treated cells (Figure 1, bottom trace) shows only a very weak carotenoid absorption, peaking around 482 nm, whereas BChl absorption maxima are now located at 889, 802, 591 and 380 nm.

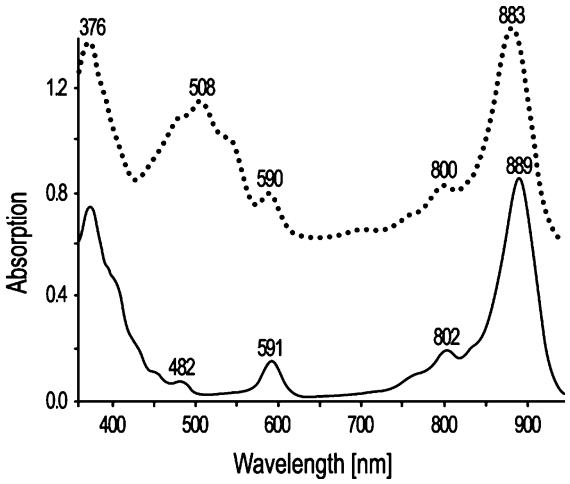

Figure 1. Absorption spectra of core complexes from normally grown *Thiorhodospira sibirica* strain A12^T (• • • •) and the bacterium grown in the presence of DPA (——).

The most striking feature associated with the absence of carotenoids in the core complex, is a red shift of the LH1 Q_y transition from 883 nm (native) to 889 nm (carotenoid-deficient). No such shifts were seen in DPA grown cells of *Rsp. rubrum* (Malhorta et al. 1970) and Crt-depleted core complexes from *Chr. minutissimum* (Toropygina et al. 2003) and *Ect. haloalkaliphila* (Moskalenko et al. unpublished). Instead, significant (10–12 nm), but opposite (= blue) shifts were observed in practically all other carotenoid-less variants of LH1 and core complexes (Miller et al. 1987; Chang et al. 1990; Davis et al. 1995; Loach and Parkes-Loach 1995). Obviously, complex-bound Crts affect the electronic transitions of the excitonically coupled BChl, but the molecular basis remains unclear (Davis et al. 1995). Recent results with model LH1 complexes indicate that direct carotenoid–BChl interactions are not involved (Fiedor et al. 2004).

Interactions of core complex with detergent

The Crt-deficient core complex from the inhibited cells of *Trs. sibirica*, treated with 20% β-OG at room temperature, dissociates completely within 30–240 min, depending on the molar ratio of detergent to the complex. The dissociation of native (normal Crt content) core complex under

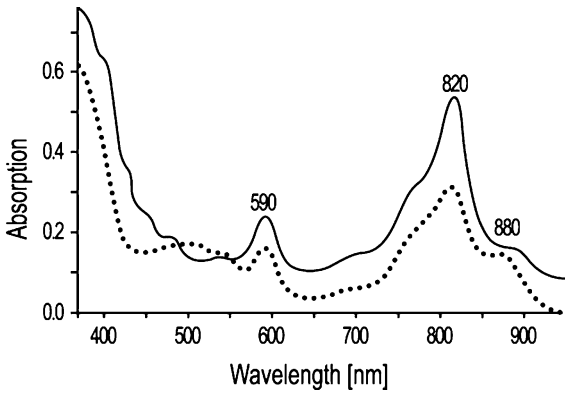

Figure 2. Detergent-induced changes in the absorption spectra of core complexes from normally grown *Thiorhodospira sibirica* strain A12^T (• • • •), and from the DPA-inhibited bacterium (——).

the same conditions is slower and usually does not proceed to completion. In both cases, during the detergent treatment a decrease of the maximum near 880 nm and an increase in the absorption at 820 nm and near 770 nm are observed (Figure 2). Apparently, the detergent causes a dissociation of the core antenna into the B820 subcomplex, which further dissociates into smaller subunits and/or free pigments (Miller et al. 1987; Ghosh et al. 1988; Chang et al. 1990; Jirsakova and Reiss-Husson 1993). The native antenna dissociation is accompanied by a large decrease of the Crt absorption, similar to that observed during the B880 → B820 transformation in photosynthetic membranes of *Rsp. rubrum*, which has been explained by Crt release and its irreversible precipitation (Moskalenko et al. 1995; Agalidis et al. 1999). During the dissociation of the Crt-deficient core complex no release of Crts is observed and the residual Crts seem to remain bound within the complex (Figure 2, upper trace).

The pigment–protein complexes resulting from the dissociation of the core complexes were analyzed by native PAGE and in parallel, by ion exchange chromatography on DEAE-Sepharose, both performed in the presence of 0.8% β-OG. The detergent-treated Crt-depleted core complex resolves into two pigmented electrophoretic fractions; the native core complex yields an additional one of intermediate mobility (Figure 3A). Ion exchange chromatography proved harsher: only the B820 subcomplex fraction could be clearly resolved, and a fraction of free BChl was observed on top of the column (not shown).

The absorption spectra of the electrophoretic fractions, taken directly in the native gels, are shown in Figure 4. The spectra of the slowest migrating bands (a) have three absorption maxima in the long wavelength region, at 760, 800 and near

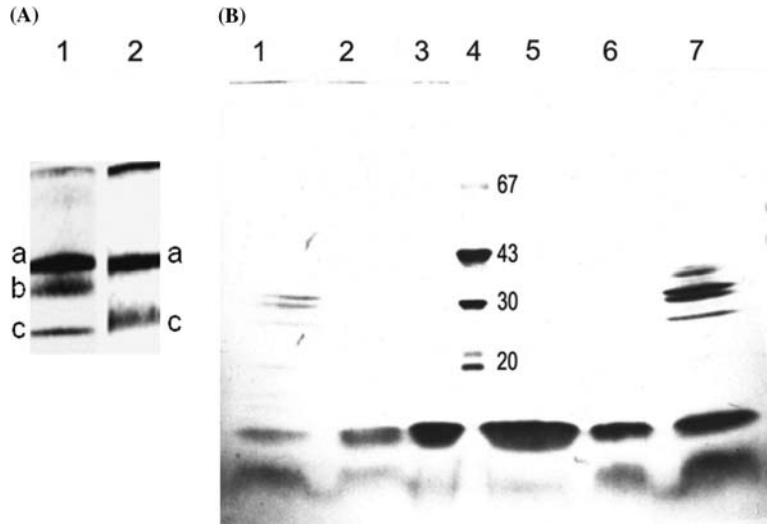

Figure 3. (A) Native PAGE (0.8% β-OG) of core complexes from non-inhibited and DPA-inhibited *Thiorhodospira Sibirica*; (B) subsequent LDS-PAGE analysis of polypeptide composition of the resulting B820 subcomplexes and RC-LH1$_{fr}$ complexes (see the text for details). Lane assignments: (A) Complexes from 1: non-inhibited, 2: DPA-inhibited cells (a: RC-LH1$_{fr}$ complex; b: unidentified intermediate; c: B820 subcomplex); (B) 1 and 7: electrophoretically isolated LH1$_{fr}$-RC, from non-inhibited and inhibited cells, respectively; 2 and 6: electrophoretically isolated B820, from non-inhibited and inhibited cells, respectively; 3 and 5: B820 isolated by ion exchange chromatography, from non-inhibited cells; 4: marker proteins (mw given in kDa).

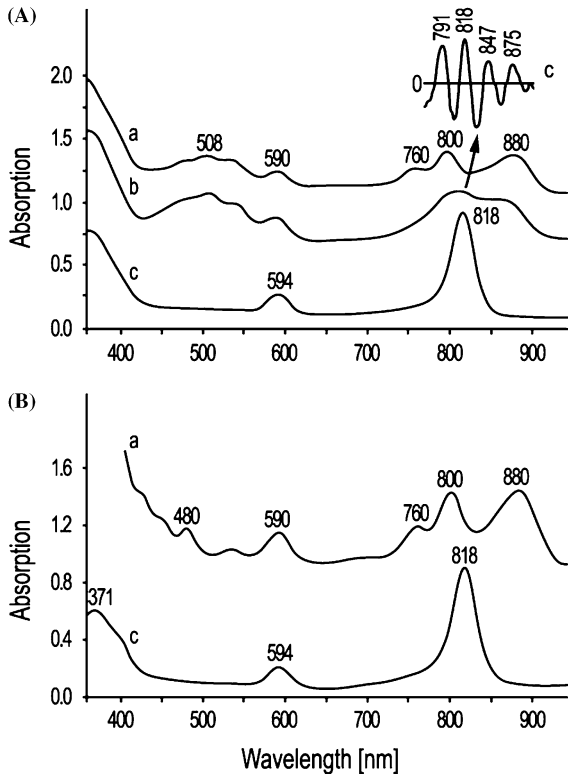

Figure 4. Spectroscopic analysis of products of 20% β-OG treatment of core complexes from normally grown *Thiorhodospira sibirica* strain A12^T (top), and the DPA-inhibited bacterium (bottom), separated by PAGE under native conditions (see the text for detail). The absorption spectra were measured *in situ* from the bands in the PAGE gel shown in Figure 3A; the order and labeling of the spectra corresponds to those of the bands in Figure 3. Inset: 4th derivative of trace 6 from the upper panel, calculated for the region between 750 and 900 nm.

880 nm, which resemble somewhat the absorption profile of RC overlapped with an additional absorption at 880 nm. Another indication of the RC presence in these fractions is their photochemical activity, determined using a phosphoroscope (not shown). The polypeptide analysis of the two bands by lithium dodecyl sulfate (LDS)-PAGE (Figure 3B) indeed reveals proteins of molecular masses (of approximately 25, 28 and 30 kDa), closely matching the ones of the RC components, and also significant amounts of a protein in the region of low molecular mass, between 6 and 10 kDa. The presence of the latter, if associated with the absorption band at 880 nm, points to the presence of LH1 antenna together with the RC in this band. There are several arguments why the presence of this residual antenna cannot be explained as an admixture of intact LH1 complex accidentally comigrating with the RC: (1) prior to PAGE, the core complexes were subjected to rather harsh dissociative treatment with a concentrated detergent, destructive in particular to the Crt-devoid LH1 (Ghosh et al. 1988); (2) intact antenna (or core complex) is expected to be of different electrophoretic mobility (see Figure 3A); and (3) the absorption profile is reproducible while a chance mixture of LH1 and RC would yield different spectra in each preparation. Therefore, this slowest migrating species seems to represent specific and stable associates of RC with fragments of LH1 antenna ($LH1_{fr}$) and was consequently termed RC–$LH1_{fr}$. Complexes with even more reduced LH1 contents (RC + 1 or 2 $[BChl]_2/\alpha\beta$ heterodimers) have been already observed upon detergent treatment of the core complex of *Rsp. rubrum* (Moskalenko et al. 1995; Ghosh, private communication).

The absorption profiles of the two (noninhibited and DPA-inhibited) RC–$LH1_{fr}$ complexes are similar in the long wavelength region but show significant differences in the blue part of the spectrum (Figure 4). As expected, the non-inhibited core complex yields RC–$LH1_{fr}$ complex with normal Crt complement, whereas after inhibition the Crt absorption is strongly reduced and appears blue-shifted (480 nm), as in the spectrum of the original DPA-inhibited core complex (Figure 1, bottom trace). The short wavelength position of this band points to the presence of Crts with a shorter conjugation system, such as methoxyneurosporene and ζ-carotene, in agreement with the results of the pigment analysis by HPLC (Table 1).

Interestingly, after the dissociating detergent treatment of the DPA-inhibited core complex, the residual Crts are found always with the RC–$LH1_{fr}$ complex. Although the A_{800}/A_{Crt} ratio in this complex resembles that found e.g. in the RC from the wild type *Rba. capsulatus* (Friesner and Won 1989), there are no indications as to the location of these Crts within the complex. For instance, their binding in the RC would imply their 15-*cis* conformation (Koyama and Fujii 1999), which was not confirmed at present.

The fastest migrating bands (c), obtained from the detergent treated native and the carotenoid-depleted core complexes, also have similar electrophoretic mobilities and absorption spectra. Both fractions can be identified as B820 subcomplexes, based on the positions of their absorption maxima

Table 1. Carotenoid composition of RC–LH1 core complexes isolated from normally grown and DPA-treated *Thiorhodospira sibirica*, determined by HPLC on reversed-phase silica (nd = not detected)

Carotenoid	Before inhibition (% of total)	After inhibition (% of total)	ϵ_{max} (M^{-1} cm^{-1})	Ref.
Spirilloxanthin	33.9	nd	147,200	(Britton et al. 2004)
Rhodopin	15.9	trace	166,000	(Davies and Köst 1988)
Anhydrorhodovibrin	36.3	nd	153,000	(Britton et al. 2004)
Lycopene	13.7	nd	185,000	(Davies and Köst 1988)
Derivative of OH-Methoxyneurosporene	nd	8.1	157,000	(Britton et al. 2004)
OH-Methoxyneurosporene	nd	24.5	157,000	(Britton et al. 2004)
ζ-Carotene	nd	64.3	143,000	(Britton et al. 2004)
OH-ζ-Carotene	nd	3.1	143,000	(Britton et al. 2004)

at 818, 594 and 371 nm (Figure 4), and according to the polypeptide analyses of these fractions by LiDS-PAGE, which revealed only two polypeptides of low molecular weight (Figure 3B). The same type of complex was obtained also by ion exchange chromatography of the detergent-dissociated core complexes. Its circular dichroism spectrum (not shown) with a slightly non-conservative pattern with a positive lobe centered near 780 nm and a negative one centered near 820 nm, is identical to the spectrum reported previously (Parkes-Loach et al. 1988; Visschers et al. 1992) and supports this assignment.

The *in situ* recorded absorption spectrum of the band (b) of intermediate mobility has four poorly resolved bands located at 791, 818, 847 and 875 nm (Figure 4, top), as determined from the fourth derivative spectrum (Figure 4, inset). The four absorption bands could be reproducibly identified in different preparations, but with variable intensities. There are no obvious absorption maxima which could be assigned to the RC and also no photochemical activity was observed, but RC proteins were detected along with the LH1 polypeptides in this fraction by LiDS-PAGE electrophoresis (not shown). The origin of this intermediate species is difficult to explain. It might be simply a result of comigration of various fragments of the RC and antenna complexes, which are likely to be formed during the harsh detergent treatment of the core complex. In spite of the fact that the absorption bands near 820 and 850 nm are usually associated with carotenoidless subforms of LH1 complex (Miller et al. 1987; Chang et al. 1990; Pandit et al. 2003), the Crts present in this fraction still seem to be involved in strong interactions (Fiedor et al. 2004), at least according to the positions of their absorption maxima (Figure 4, top), as red-shifted as in the intact core complex (compare with Figure 1, upper trace).

The dissociation of the core complex and its interactions with detergent can be summarized as follows: the core complex in its native environment (photosynthetic membranes) is relatively stable towards β-OG; the treatment of chromatophores does not lead to its fragmentation. The core complex with the normal Crt content is particularly stable towards this detergent. The assembly becomes more vulnerable after a pretreatment (solubilization) with DM and subsequent separation by preparative electrophoresis. The treatment with DM probably (partially) solubilizes the membrane lipids and removes some lipid components from the surface of the core complexes, which contribute to the stability of the assembly in its native state. These exposed areas may then be the sites for further detergent action. It is likely that the fragmentation begins by loss of Crts from the antenna into the detergent micelles; this lowers the antenna stability and free B820 subunits are being released. Eventually, as most of the subunits are stripped off, the core complex is cut down to a fragment RC-$LH1_{fr}$ of relatively higher stability.

The photosynthetic bacteria can be induced to produce photosynthetic membranes with much reduced Crt levels, e.g. by using inhibitors of Crt biosynthesis. The core complexes from such cells are considerably destabilized: dissociation into the B820 subunits occurs more easily than in the presence of native Crts. In spite of this instability,

some antenna fragments still remain bound to RC and form a stable RC-$LH1_{fr}$ complex, apparently stabilized by the residual Crts.

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