Light-Harvesting Complexes of Aerobic Bacteriochlorophyll-Containing Bacteria

*Roseococcus thiosulfatophilus*, RB3 and *Erythromicrobium ramosum*, E5 and the Transfer of Excitation Energy from Carotenoids to Bacteriochlorophyll

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Introduction

*Roseococcus thiosulfatophilus*, RB3 (Yurkov and Gorlenko, 1992) and *Erythromicrobium ramosum*, E5 (Yurkov et al., 1991, 1992) are obligate aerobic chemotrophic bacteria which can synthesize a photosynthetic apparatus. The contribution of light energy to the formation of an electrochemical proton gradient across the cytoplasmic membrane in these bacteria is not known, but seems to be low (Harashima et al., 1987; Yurkov and van Gemerden, 1993). The reasons why these bacteria can not grow photosynthetically under anaerobic conditions as the photosynthetic purple bacteria are unknown.

Many strains of these aerobic, bacteriochlorophyll (BChl)-containing bacteria belong taxonomically to the α-subclass of *Proteobacteria* (Woese, 1987; Harashima et al., 1988; Shiba et al., 1991; Fuerst et al., 1993; Yurkov et al., 1994). Recently it was shown that the DNA sequence of the operon, encoding the genes for the proteins of the *puf* operon, encoding the genes for the proteins of the light-harvesting complex I (LH I), the reaction center (RC) and the tetraheme subunit of *Roseobacter denitrificans*, is very similar to that of *Rhodobacter* species (Liebetanz et al., 1991).

The organization of photosynthetic RC seems to be similar to that of purple bacteria. Two types of LH antenna systems are present (Harashima et al., 1988). They were named LH I (B870) and LH II (B800–850) according to the terminology used for purple bacteria although some of them have slightly different absorption bands.

In a previous article we have shown that cells of *R. thiosulfatophilus* and *E. ramosum* contain a large number of unusual and mostly polar carotenoids (Crt) (Yurkov et al., 1993).

The molar ratio of Crt to BChl was found to be 9:1 in whole cells but in LH complexes 0.1 to 1:1. Bacteriorubixinthinal was the major Crt in the pigment proteins of the LH and RC complexes of *E. ramosum*. In addition small amounts of spirilloxanthin (LH I + RC) and zeaxanthin (LH II) were present. In *R. thiosulfatophilus* C30-carotene-dioate (4,4′-dioate) (compound A) and the respective diglucosyl ester (compound B) are the major carotene compounds. The enriched LH I + RC fraction contains mainly compound B (di-(β-D-glucopyranosyl)-4,4′-diapocaro-
tene-4,4’-dioate). The Crt’s which are not bound to the BCHl-protein complexes, were found in the cell envelope fraction (cytoplasmic membrane and outer membrane) (Yurkov et al., 1993). The function of these carotenoids is unknown. They may play a role in scavenging free radicals and/or singlet oxygen, processes which have been observed for several Crt’s in organic solvents (Krinsky, 1979, 1989; Oliveros et al., 1992) or they screen the cells from high light intensities.

Materials and Methods

Strains, cultivation and membrane isolation

Roseococcus thiosulfatophilus strain, RB3, DSM N8511, and Erythromicrobium ramosum, E5, DSM N8510, were cultivated at 30 °C semi-aerobically in the dark in order to maximize the formation of the photosynthetic apparatus. The bacteria were grown on a yeast extract–peptone–acetate medium (Yurkov et al., 1993) in a 12 l New Brunswick fermenter (stirring at 200 rpm) for 48 h. Cell-harvesting, disruption in a French pressure cell, and fractionation of cell membranes has been described previously (Yurkov et al., 1993).

Isolation of pigment-protein complexes

Membrane fractions I and II of R. thiosulfatophilus and fraction I of E. ramosum, isolated by sucrose density gradient centrifugation (Yurkov et al., 1993), were used for spectroscopical studies and for isolation of pigment-protein complexes. These membrane fractions (about 17 µg BCHl/ml) were resuspended in 20 mM Tris-HCl (pH 7.8) buffer and mixed (1:1) with 1.2% of lauryldimethylamine N-oxide (LDAO). The suspension was incubated at room temperature in the dark under gentle stirring. After 30 min the mixture was layered on a sucrose step gradient (0.3, 0.6 and 1.2 M sucrose in Tris-HCl buffer of 20 mM, pH 7.8) containing 0.05% LDAO. After centrifugation for 16 h at 90,000×g in a Beckman Ti60 rotor the pigmented protein bands were collected.

For further purification the LH II- and LH I-enriched fractions were directly applied to a DEAE-Sepharose column equilibrated with 20 mM NaPO₄ buffer (pH 8.0).

The complexes were purified by stepwise elution with increasing concentrations of NaCl in 20 mM NaPO₄ buffer (pH 8.0), containing 0.05% LDAO.

Analytical methods

BCHl and Crt were extracted from whole cells, membrane fractions and pigment-protein complexes with acetone:MeOH 7:2 (v/v). The total amount of BCHl was determined according to Clayton (1966). The protein content was measured by the method of Lowry et al. (1951). Membrane proteins were separated by SDS-PAGE on an 11.5–16.5% linear gradient of acrylamide. Proteins were stained with Coomassie Brilliant Blue, G-250.

Spectroscopic methods

Absorption spectra at room temperatures were recorded with a Kontron spectrophotometer UVICON 860, and plotted by using the computer program “Diagramme” (Atari). Absorption, fluorescence and fluorescence excitation spectra at low temperatures were recorded using a home-built spectrometer, described in detail in Kaiser et al. (1981), Kaiser (1982) and Angerhofer et al. (1986).

The quantum yield of energy transfer from Crt’s to BCHl was calculated from the intensities of their respective absorption bands in the absorption and fluorescence excitation spectra according to the following formula (Goedheer, 1959):

\[ \eta = \frac{I_{\text{exc}}^{\text{Crt}} \times I_{\text{abs}}^{\text{BChl}}}{I_{\text{exc}}^{\text{Crt}} \times I_{\text{exc}}^{\text{BChl}}} \]

\( \eta \) is the quantum yield of the energy transfer from the Crt excitation band (\( I_{\text{exc}}^{\text{Crt}} \) is the intensity of the excited Crt absorption band in the excitation spectrum, \( I_{\text{abs}}^{\text{BChl}} \) – the intensity of the same band as found in the absorption spectrum) to the fluorescence first singlet state of the BCHl (\( I_{\text{exc}}^{\text{abs}}^{\text{BChl}} \) – are the intensities of the \( S_0 \rightarrow S_1 \) fluorescence excitation and absorption bands of BCHl). The intensities may be taken just from the peak values of the spectra since differences in linewidth between corresponding peaks in the absorption and fluorescence excitation spectra were not observed. Additionally, we applied a multi-Gaussian fit to the spectra and extracted the area under the respective excitation bands for the calculation of the quantum yields. Both methods gave compa-
rable results to within ±5% in all cases. In the following the average values gained by both methods will be given. Calculation of band intensities was done with spectra corrected for the instrument sensitivities and plotted on a linear photon energy scale (i.e. in cm⁻¹).

Results

*Roseococcus thiosulfatophilus, RB3, pigment-protein complexes*

From detergent-treated membranes of *R. thiosulfatophilus* a BChl-protein complex was isolated (see Methods) and eluted from DEAE-Sepharose columns at 350 mM NaCl. The complex showed a main absorption band at 856 nm (at room temperature; Fig. 1a). Since the absorption spectrum of this complex in the near-infrared (NIR) region was almost identical to that of the membrane (Fig. 1 in Yurkov et al., 1993) and little BChl was lost during the isolation procedure, most if not all of the BChl molecules in strain RB3 seems to be organized in this complex.

The NIR absorption maximum of the membrane fraction appearing at 864 nm at 6 K (see Fig. 3a, b-II) is unusual for purple bacteria because LH I complexes normally show maxima between 870 and 890 nm. The ratio of Crt to BChl absorption is relatively high (see Fig. 1b in Yurkov et al., 1993, and Fig. 3a, b-II). The peaks at 484 nm, 514 nm, and 557 nm are tentatively assigned to

![Absorption spectra](image)

*Fig. 1. Absorption spectra of isolated pigment-protein complexes recorded at room temperature. a) LH I + RC complex isolated from *Roseococcus thiosulfatophilus*; b) LH I + RC complex isolated from *Erythromicrobium ramosum*; c) LH II complex isolated from *E. ramosum.*

![Protein patterns](image)

*Fig. 2. Protein patterns of membranes or pigment-protein complexes separated by SDS polyacrylamide gel electrophoresis. a) Isolated from *Roseococcus thiosulfatophilus*, lane A: protein markers in kDa; lane B: membrane fraction from *Rhodobacter capsulatus* 37b4, from top to the bottom LH IIγ, LH Iα, LH IIα, LH IIβ and LH Iβ, double band; lane C: membrane fraction I of *R. thiosulfatophilus*; lane D: membrane fraction II of *R. thiosulfatophilus*; lane E: LH I-enriched fraction from membranes of *R. thiosulfatophilus*. b) *Erythromicrobium ramosum*, lane A: membrane fraction; lane B: enriched LH I complex; lane C: LH II complex; lane D: molecular weight markers in kDa.*
compound B (see Introduction). They appeared with lower intensity in fraction II of the membrane (Fig. 3b) compared with fraction I (Fig. 3a). Fraction II showed a lower Crt/BChl ratio than fraction I (10:1).

The LH I + RC complex showed the same Crt absorption maxima as the membrane but a lower Crt/BChl ratio of about 1.4:1.0 (Fig. 3c-II), with approximately three times less Crt’s per BChl (mol:mol) than membrane fraction I.

The polypeptide patterns of the isolated LH I complex was analyzed by SDS-PAGE (Fig. 2a). Four polypeptides with an apparent Mr of about 8000 (two upper) and 7000 (two lower) were observed. These polypeptides were also present in membrane fractions of R. thiosulfatophilus (Fig. 2a). The complex is named LH I.

Fluorescence and quantum yield of Car $\Rightarrow$ BChl singlet energy transfer

The quantum yield determination of excitation energy transfer are based on the total Crt’s present in the respective fractions since we could not obtain highly purified RC and LH I preparations. The fluorescence emission spectrum of membrane fraction I showed one peak at 876 nm, originating from the LH I emission (see Fig. 3a-I). The main peak of the absorption and fluorescence excitation spectra (Fig. 3a-III) was at 864 nm. In the visible region the relative intensities differ considerably. The strong Crt absorption bands at 484, 514 and 557 nm (Fig. 3a-II) contributed only weakly to the fluorescence excitation spectrum 499, 571 nm; Fig. 3a-III). These data support the idea that only a subpopulation from the bulk of the Crt’s in the membranes is efficient in energy transfer to LH I. The quantum yield calculated for the total Crt content was 7.7%. If we assume a Crt/BChl ratio of 1:1 in the LH I complex and neglect energy transfer from the membrane-bound secondary Crt’s (Crt/BChl ratio 9:1) for membrane fraction I, one can estimate the quantum yield of Crt $\Rightarrow$ BChl energy transfer in the native LH I complex to 77%, well in the range of values observed in purple photosynthetic bacteria (Noguchi et al., 1990).

The spectral location of the fluorescence emission and excitation and absorption bands in fraction II are the same as in fraction I (Fig. 3b), but the Crt absorption bands are weaker than in fraction I. A quantum yield for energy transfer of 11.2% was derived. This, however, reflects a lower concentration of bulk carotenoids (inactive in energy transfer to BChl) in this membrane preparation, rather than a higher energy transfer efficiency for the protein-bound carotenoids.

The isolated LH I + RC complex of R. thiosulfatophilus displayed the same fluorescence emission peak at 876 nm as in membrane fractions I and II (Fig. 3c-I). The fluorescence excitation and absorption spectra have a NIR band at 864 nm. At 594 nm the Qx band of the BChl is visible in the excitation spectrum. The Crt bands appeared in the excitation spectrum at 495, 528 and 569 nm which are somewhat red-shifted compared to the
peak maxima in the absorption spectrum. From the intensities a quantum efficiency for energy transfer of 29% was calculated. The fact that the Crt excitation bands were not found at the same spectral positions in both spectra may indicate that RC and LH complexes have different carotenoids. The quantum efficiency calculated that way was then obscured by the RC Crt’s which do not contribute to the LH fluorescence.

Erythromicrobium ramosum, E5, pigment-protein complexes

From membrane fraction I of *E. ramosum* (Yurkov *et al.*, 1993) two subfractions were obtained after treatment with LDAO and sucrose density gradient centrifugation. The upper band was enriched in LH II and the lower band in LH I + RC. Both fractions were abundant in Crt’s. The bands were separately applied to DEAE-Sephrose chromatography. The LH I + RC complex was eluted at 225 mM NaCl, the LH II at 100 mM NaCl. At room temperature the purified LH I + RC complex showed a main absorption band at 871 nm and a smaller one at 801 nm (Fig. 1b). LH II absorbed at 798 nm and 832 nm (Fig. 1c). At 6 K absorption maxima at 440 nm, 467 nm and 497 nm in the Crt absorption region, and at 589 nm, 797 nm, 838 nm and 878 nm, belonging to BChl were observed in the membrane fractions, indicating the presence of two LH complexes (Fig. 4a).

The isolated LH II complex (Fig. 4b) showed BChl maxima at 796 and 840 nm (BChl) and Crt maxima at 503 nm and 535 nm indicating a change in the Crt composition compared with the whole membranes. Für LH I + RC fraction showed a LH I maximum at 884 nm (Fig. 4c) and a very weak Crt band around 500 nm.

Both LH II and LH I + RC fractions contained bacteriorubixanthinal as the main Crt compound and additionally zeaxanthin in the LH II fraction and spirilloxanthin in the LH I + RC fraction (Yurkov *et al.*, 1993).

The LH I complex contained two polypeptides with an apparent molecular weight of about 8000 and 6000 and the LH II preparation three polypeptides of about 16,000, 9000 and 8000 Mr. The RC bands have not been identified.

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**Fig. 4.** Absorption (II), fluorescence emission (I) and fluorescence excitation (III) spectra of fraction isolated from *Erythromicrobium ramosum* recorded at 6 K. a) Membrane fraction; b) light-harvesting complex II (LH II); c) LH I + RC fraction. Absorption and fluorescence excitation spectra are displayed normalized to equal height of their NIR band. The respective fluorescence spectra appear normalized to the height of this band. Fluorescence and fluorescence excitation spectra were corrected to the instrumental response function.

**Fluorescence and quantum yield of Car => BChl singlet energy transfer**

The fluorescence emission bands of the membrane fraction of *E. ramosum* (Fig. 4a-I) at 896 nm and a weaker one at 859 nm, at 6 K, are indicative of LH I and LH II fluorescence, respectively. The fluorescence excitation spectrum taken at a detection wavelength of 897 nm showed the same spectral features as the absorption spectrum. Both spectra were normalized to the same intensity of the NIR band at 878 nm of the LH I complex. The two bands at 797 nm and 838 nm belong to the LH II complex. The intensity of these bands was about 30% lower than in the corresponding absorption spectrum, indicating that energy from
LH II to LH I was transferred at a low rate. This also explains why a LH II fluorescence emission band at 859 nm was observed. In the visible region the BChl Qx band was visible at 589 nm and the Crt absorption bands at 440, 467, 497 nm and a shoulder at 532 nm.

For analysis of the quantum yield of energy transfer in the membrane fraction of *E. ramosum* the 538 nm and the 497 nm bands of the Crt’s were selected which gave yields of 25.5% and 33.0%, respectively. This difference may be explained by differences in Crt pigment composition of the LH I and LH II complexes and the Crt pool not associated with the photosystem. It also indicated that the Crt’s not associated with the LH proteins are inactive in energy transfer to the BChl bound to these proteins.

The fluorescence emission spectrum of the LH II complex showed only one band with its maximum at 867 nm (Fig. 4b). The absorption and fluorescence excitation spectra revealed peaks at 503, 535, 586, 796, and 840 nm. The quantum yield of Crt to BChl energy transfer was calculated to be 94%. The fluorescence emission spectrum of the LH I + RC preparation (Fig. 4c) was characterized by one main peak at 915 nm, and two weak peaks at 861 nm and 790 nm. The main band was ascribed to the LH I emission. The weaker maxima at shorter wavelengths were probably due to residual LH II emission and free BChl. The fluorescence excitation and absorption spectra were again very similar with respect to the position of their peaks. They differ in intensity only in those bands which must be ascribed to the RC. The main band in the NIR appeared at 884 nm with a small shoulder at 838 nm, probably due to the residual LH II complex. The bands at 763 and 796 nm, belonging to the RC, were weaker by at least 50% in the fluorescence excitation spectrum. These results indicate some energy transfer from RC’s to the LH I pool. In the visible region the BChl Qx band at 590 nm and weak Crt bands at 498 nm and 533 nm were observed. From their intensities a quantum yield of 60% was calculated.

**Discussion**

The species described in this article are not closely related to *Rhodobacter capsulatus* or *Rhodobacter sphaeroides* (Yurkov et al., 1994). The LH complexes of these bacteria differ from those of many purple bacteria. The major NIR absorption band of the LH I + RC-enriched fraction of *Rhodopseudomonas acidophila* and *R. palustris* (Bissig et al., 1990; Evans et al., 1990; Gardiner et al., 1992; Tadros et al., 1993). The isolated LH II complex contained three proteins like *R. capsulatus*.

In previous studies a RC-B865 complex from *Erythrobacter longus*, OCh101, and RC-B870 and B806 complexes from *Roseobacter dentifricans*, OCh114 were isolated and described (Shimada et al., 1985). The isolation of new types of LH complexes, described in this article, underlines that these aerobic bacteria are heterogenous in their physiology, taxonomic position and composition of the photosynthetic apparatus.

The emission and excitation spectra of the membrane fractions of both *R. thiosulfatophilus* and *E. ramosum* demonstrate that most of the membrane-bound Crt’s are not associated with the LH or RC complexes, and that these separately localized Crt’s do not transfer excitation energy to the RC. The quantum yields for excitation energy transfer are thus only lower estimates to the true quantum yields in the LH complexes. This is also true for the isolated LH I + RC complexes of *R. thiosulfatophilus* and *E. ramosus* since the Crt’s of the RC do not transfer their excitation energy to the LH I complex. The quantum yields of 29% and 60%, respectively, are thus only lower bounds of the true quantum yields in these LH I complexes.

The quantum yield of energy transfer was more exactly determined for the LH II complex of *E. ramosum*. A quantum yield of 94% is comparable to what has been observed for the B800–850 complex of the purple bacterium *Rhodobacter sphaeroides* strain 2.4.1 (Cogdell et al., 1981; van Grondelle et al., 1982; Hayashi et al., 1987; Nogu-
chi et al., 1990), but higher than the yields observed for the LH II complexes of other purple bacteria (Angerhofer et al., 1986; Noguchi et al., 1990; Cogdell et al., 1992).

High concentrations of Crt's not active in photosynthetic energy transfer have been observed in membranes of Erythrobacter longus, OCh 101 (Noguchi et al., 1992). The main Crt was (3S, 2'R,3'R)-3,2',3'-trihydroxy-ß,ß-carotene-4-one-3-sulphate (erythroxanthin sulphate), a highly polar molecule (Takaichi et al., 1991). It was suggested that its possible function is photoprotection by quenching harmful singlet oxygen (Noguchi et al., 1992). High concentrations of "extrinsic" carotenoids have also been observed in algae. It has recently been suggested that high extrachloroplastic concentrations of astaxanthin ester-containing lipid globules function as a sunscreen to minimize photodamage during periods of exposure to intense solar radiation of snow algae (e.g. Chlamydomonas nivalis; Bidigare et al., 1993). In addition to the "sunscreen" effect these secondary Crt's may act as inhibitors of photodynamically induced damage as indicated by a recent study of Hagen et al. (1993) on Haematococcus lacustris.

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