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Low-temperature fluorescence and absorption spectroscopy of reaction center/antenna complexes from *Ectothiorhodospira mobilis*, *Rhodopseudomonas palustris* and *Rhodobacter sphaeroides*

Tina Leguijt [a], Ronald W. Visschers [b], Wim Crielaard [a], Rienk van Grondelle [b] and Klaas J. Hellingwerf [a]

[a] *Department of Microbiology, University of Amsterdam, Amsterdam and* [b] *Department of Biophysics, Free University of Amsterdam, Amsterdam (Netherlands)*

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Reaction centers (RC's) with either one or both light-harvesting complexes attached (RC/LH_I- or RC/LH_I/LH_{II} complexes), have been isolated from the photosynthetic purple bacteria *Ectothiorhodospira mobilis*, *Rhodopseudomonas palustris* and *Rhodobacter sphaeroides*, using ionic and non-ionic detergents. The isolated complexes were analyzed with respect to their physical and functional properties using low-temperature (77 K) absorption, circular dichroism and fluorescence spectroscopy. The stability of the association between the antenna complexes and the RC, and consequently the functional interaction of the RC/antenna complexes after isolation, differed among the bacteria investigated in this study, particularly with respect to detergent sensitivity. By using the non-ionic detergent octyl glucoside we were able to isolate functionally intact RC/antenna complexes from *E. mobilis*, while for the isolation of these complexes from *Rps. palustris* and *Rb. sphaeroides* the ionic detergent cholate had to be used. In the RC/antenna complexes from *E. mobilis*, the energy transfer between LH_{II} and the core antenna was sensitive to detergents, as judged by an increase of the LH_{II} fluorescence at 77 K. A strong increase in the LH_I and LH_{II} fluorescence in RC/antenna complexes isolated with octyl glucoside from *Rb. sphaeroides*, indicated that in this organism the functional interaction between both LH_{II} and LH_I, as well as between LH_I and the RC was easily disrupted. In *Rps. palustris*, the functional association between the antenna complexes and the RC was stronger compared to the other two investigated species.

Introduction

The photosynthetic apparatus of purple bacteria is composed of several light-harvesting (antenna) pigment-protein complexes that surround and interconnect the membrane-spanning reaction centers (RC's) [1], and resides in specialized domains of the cytoplasmic membrane [2]. The light-harvesting complexes are composed of bacteriochlorophyll (BChl) and carotenoids, which are spatially arranged by small polypeptides (see Ref. 3 for a review). Light energy is absorbed by the carotenoids and BChl's and the energy is rapidly transferred from the light-harvesting complexes to the photochemical reaction center, where charge separation can take place (see Ref. 4 for a recent review).

The antenna complexes of the purple bacteria can genetically and physiologically be divided into two major types: (i) a core complex antenna (LH_I or B875; B880; B890), which is shown to be in final contact with the RC and always present in a fixed LH_I/RC stoichiometry [5], and; (ii) a peripheral antenna complex (LH_{II} or B800/850), which is associated with the core

Correspondence to: K.J. Hellingwerf, Department of Microbiology, University of Amsterdam, Nieuwe Achtergracht 127, 1018 WS Amsterdam, Netherlands.

Abbreviations: BChl, bacteriochlorophyll; B875/880/890, light-harvesting complex I; B800/850, light-harvesting complex II; cholate-RC/LH_I/LH_{II}, RC/LH_I/LH_{II} complexes isolated with 1% cholate; CD, circular dichroism; $A_{(905)}$, absorbance (at 905 nm); $LH_{I/(II)}$, light-harvesting complex I or II; NIR, near-infrared region; octyl glucoside, *n*-octyl β-D-glucopyranoside; octyl glucoside-RC/LH_I/LH_{II}, RC/LH_I/LH_{II} complexes isolated with 30 mM octyl glucoside; PMS, *N*-methyl phenazonium methosulfate; RC, reaction center.

complex and synthesized in variable amounts. Both types of light-harvesting complex can be found in representatives of both purple sulfur (e.g., *Ectothiorhodospira mobilis*) and purple non-sulfur bacteria (e.g., *Rhodopseudomonas palustris* and *Rhodobacter sphaeroides*).

In *Rb. sphaeroides* both LH_I and LH_{II} are composed of two different polypeptides, the α- and β-subunits, which are present in an 1 : 1 ratio (see Ref. 6 for a recent review). The antenna polypeptide composition of *Rps. palustris* is more heterogenous. The individual complexes in this organism appear to be composed of at least two different α- and β-polypeptides. For some complexes (i.e., in low-light LH_{II} complexes) they consist of four α- and β-polypeptides in a molar ratio of 1 : 1 : 1 : 1 [7,8]. For *E. mobilis*, hardly any information is available on the pigment-binding antenna polypeptides, but in the closely related organism *Ectothiorhodospira halophila*, it has been demonstrated that the core antenna consists of two α- and two β-polypeptides. For the LH_{II} antenna of *E. halophila*, only one α-polypeptide could be detected until now [9].

Research on anoxygenic photosynthesis is predominantly focused on species belonging to the α-subdivision [10] of the purple bacteria. Much less is known about the molecular details of the photosynthetic apparatus in representatives of the *Ectothiorhodospiraceae* [11], bacteria that (in contrast to the *Chromatiaceae*) deposit elemental sulfur extracellularly (see Ref. 12 for a review). *Ectothiorhodospiraceae* normally grow under extreme saline and alkaline conditions [13]. These extreme growth conditions will enforce adaptations in the photosynthetic and bioenergetic machinery of the cells.

In this paper we describe the functional properties of isolated RC/antenna complexes from three well-characterized organisms, *Ectothiorhodospira mobilis* BN 9903, *Rhodopseudomonas palustris* NCIB 8288 and *Rhodobacter sphaeroides* 2.4.1. These latter two organisms were not only chosen for comparison, but also because a detailed spectroscopic analysis of RC/antenna complexes from these organisms (isolated with the aim of reconstitution of these complexes into liposomes), as well as investigation of the effect of mild detergents on the functional coupling between the antenna complexes and the RC, has not yet been performed.

The use of harsh detergents, which may disturb the delicate interaction between the antenna complexes and the RC, was avoided. Mild detergents, with a high critical micelle concentration (i.e., easily removable by dialysis), were used to solubilize RC/antenna complexes. Such complexes offer the possibility to study energy transfer in reconstituted $RC/LH_I/LH_{II}$ liposomes. Sucrose-gradient centrifugation was used to isolate the RC/antenna complexes. Either a non-ionic detergent was used, as reported for *Rps. palustris* by Molenaar et al. [14] or an ionic detergent, as reported for *Rb. sphaeroides* [15].

We report on the spectroscopic properties, energy transfer and functional interactions between the RC/antenna complexes as studied with low-temperature (77 K) absorption, CD and fluorescence spectroscopy.

Materials and Methods

Growth conditions and isolation of intracellular membranes

Ectothiorhodospira mobilis strain BN 9903 was obtained from J.F. Imhoff (Rheinischen Friedrich-Wilhelms-Universität, Bonn, Germany) and *Rhodopseudomonas palustris* strain NCIB 8288 from D.J. Kelly (University of Sheffield, UK). *Rhodobacter sphaeroides* strain 2.4.1, *E. mobilis* and *Rps. palustris* were grown anaerobically under low light intensity at 30°C. The medium for *E. mobilis* was prepared as reported previously [16]. *Rps. palustris* and *Rb. sphaeroides* were both grown in the medium as described by Lascelles [17]. Exponentially growing cells were harvested and washed twice in solubilization-buffer (A, B and C; see below for the composition of the different buffers used). Intracellular membranes were isolated according to Ref. 16. They were resuspended in solubilization-buffer (A, B or C, see below) at a concentration of 1 mM BChl *a*. These chromatophores were kept on ice until further use.

Isolation of pigment-protein complexes

Pigment-protein complexes were isolated with non-ionic and/or ionic detergents, modified from procedures as previously reported for *Rps. palustris* [14] and *Rb. sphaeroides* [15], respectively. The use of sucrose-gradient centrifugation during isolation of RC/antenna complexes yields information about the physical association between LH_{II} and RC/LH_I complexes, since both complexes band at different positions in a sucrose gradient when their association is disrupted. To isolate $RC/LH_I/LH_{II}$ complexes, with physically, as well as functionally, associated LH_{II}, we applied only the minimally required concentrations of octyl glucoside or cholate and EDTA and minimized the solubilization time.

Non-ionic. $RC/LH_I/LH_{II}$ complexes were extracted from membranes of *Rps. palustris* and *Rb. sphaeroides* with 30 mM *n*-octyl β-D-glucopyranoside (octyl glucoside) in 10 mM Tris (pH 7.6) (buffer A) plus 20 mM K-EDTA for 10 min (*Rps. palustris*) or 1 h (*Rb. sphaeroides*) at 0°C. $RC/LH_I/LH_{II}$ complexes from *E. mobilis* membranes were extracted with 30 mM octyl glucoside in 50 mM Hepes (pH 8.0), 500 mM NaCl, 5 mM $MgCl_2$, 6.5 mM Na_2S (buffer B) plus 7 mM Na-EDTA for 10 min at 0°C.

Ionic. $RC/LH_I/LH_{II}$ complexes were extracted from the membranes of *Rps. palustris* and *Rb.*

sphaeroides with 1% cholate in 50 mM potassium phosphate (pH 7.6), 50 mM KCl, 8 mM $MgCl_2$, 10% sucrose (buffer C) plus 20 mM K-EDTA for 10 min (*Rps. palustris*) and 1 h (*Rb. sphaeroides*) at 0°C. For the isolation of the $RC/LH_I/LH_{II}$ complexes from *E. mobilis*, 1% cholate in buffer B plus 7 mM Na-EDTA was used for 10 min. For the isolation of RC/LH_I complexes from this organism, a second (ionic) detergent had to be added during the extraction of the complexes from the membranes, to separate LH_{II} from RC/LH_I complexes. The extraction was performed using a combination of 30 mM octyl glucoside plus 1% sodium deoxycholate and 20 mM Na-EDTA (instead of 7 mM) for 1 h.

Solubilization and sucrose-gradient centrifugation were performed as described previously [14–16]. The isolated RC/antenna complexes were kept on ice until further use within two days.

Low-temperature spectroscopy

Samples for low-temperature spectroscopy were prepared in 66% (v/v) glycerol, diluted with the appropriate solubilization buffer. For the $RC/LH_I/LH_{II}$ complexes isolated with non-ionic detergents from *Rps. palustris* and *Rb. sphaeroides*, buffer A was used, while for *E. mobilis* buffer B was used, with omission of $MgCl_2$ and Na_2S. For the $RC/LH_I/LH_{II}$ complexes isolated with ionic detergents from *Rps. palustris* and *Rb. sphaeroides*, buffer C was used, with omission of $MgCl_2$ and sucrose. In case of *E. mobilis* buffer B was used, with omission of $MgCl_2$ and Na_2S. When cells and chromatophores of *E. mobilis* were analyzed, buffer B was used, with omission of Na_2S.

Low-temperature (77 K) measurements were performed on a home-built spectrophotometer [18]. Absorbance and fluorescence spectra at 77 K were measured in 1 cm acrylic fluorescence cuvettes. CD at 77 K was measured in 2-mm cells, as described previously [18]. Samples were cooled in the dark [18], and contained 1 mM sodium ascorbate and 10 μM *N*-methylphenazonium methosulfate (PMS) to keep the primary donor of the RC reduced. During fluorescence measurements, the absorption of the samples was kept below 0.2 to prevent distortion of spectra by self-absorption. Fluorescence-excitation spectra were corrected for spectral intensity variations of the light-source emittance. Fluorescence-emission spectra were not corrected for the spectral response curve of the photomultiplier (S1-EMI 9684), because it was essentially independent of the wavelength in the region used. All data were analyzed on a SUN3/SUN4 computer system.

Analytical procedures

BChl *a* was determined by the method of Clayton [19], using an extinction coefficient at 770 nm of 75 $mM^{-1}\,cm^{-1}$ in acetone/methanol (7:2, v/v). The RC concentration was calculated from the light-induced absorbance difference at 605–540 nm using an extinction coefficient of 37 $mM^{-1}\,cm^{-1}$ [20].

Results

Absorbance spectra

The most direct way to investigate how the properties of RC/antenna complexes are affected by different isolation procedures and/or different detergents is to follow changes in the absorbance characteristics of the various pigments involved. In the past [14,15] such studies were performed at room temperature, which has the disadvantage that the complexes display broad, partially overlapping, absorption peaks. Therefore, we decided to perform the spectral investigations at 77 K, which allows a much better comparison between native and isolated complexes.

Fig. 1A shows the near-infrared (NIR) absorbance spectra of cells, chromatophores and isolated $RC/LH_I/LH_{II}$ complexes from *E. mobilis*, taken at 77 K. It is clear that besides minor differences, all spectra were very similar with respect to peak position and relative peak intensities. In the 77 K absorbance spectrum of $RC/LH_I/LH_{II}$ complexes, isolated with cholate, a small blue-shift (1–2 nm) of the absorption peaks of B850 and B890/RC could be observed (compared to cells). In all samples the B800 displayed an absorption maximum at 795 nm (which was at the same position as the absorption peak observed at room temperature: cf. Ref. 16). In the $RC/LH_I/LH_{II}$ complexes isolated with octyl glucoside, the low-temperature absorption maxima of B850 and B890/RC were, respectively, 6 and 15.5 nm red-shifted compared to their position at room temperature (cf. Ref. 16).

In Fig. 1B the NIR absorbance spectra of $RC/LH_I/LH_{II}$ complexes isolated from *Rps. palustris* using either octyl glucoside or cholate, are shown. The 77 K absorbance spectrum of $RC/LH_I/LH_{II}$ complexes isolated with cholate, was identical to the low-temperature absorbance spectrum of intact cells (not shown). The spectra implied that the B880/RC absorption peak in $RC/LH_I/LH_{II}$ complexes isolated with octyl glucoside, has been blue-shifted.

The 77 K absorbance spectra of $RC/LH_I/LH_{II}$ complexes isolated from *Rb. sphaeroides* with either octyl glucoside or cholate, are shown in Fig. 1C. The spectrum of complexes isolated with cholate was similar to the 77 K absorbance spectrum of cells (not shown). $RC/LH_I/LH_{II}$ complexes isolated with octyl glucoside displayed a somewhat distorted spectrum, as indicated by a red-shift (5 nm) of the B875/RC absorption maximum and a small blue-shift of the B850 absorption band. No changes in the peak position of B800 were observed in the presence of the detergents

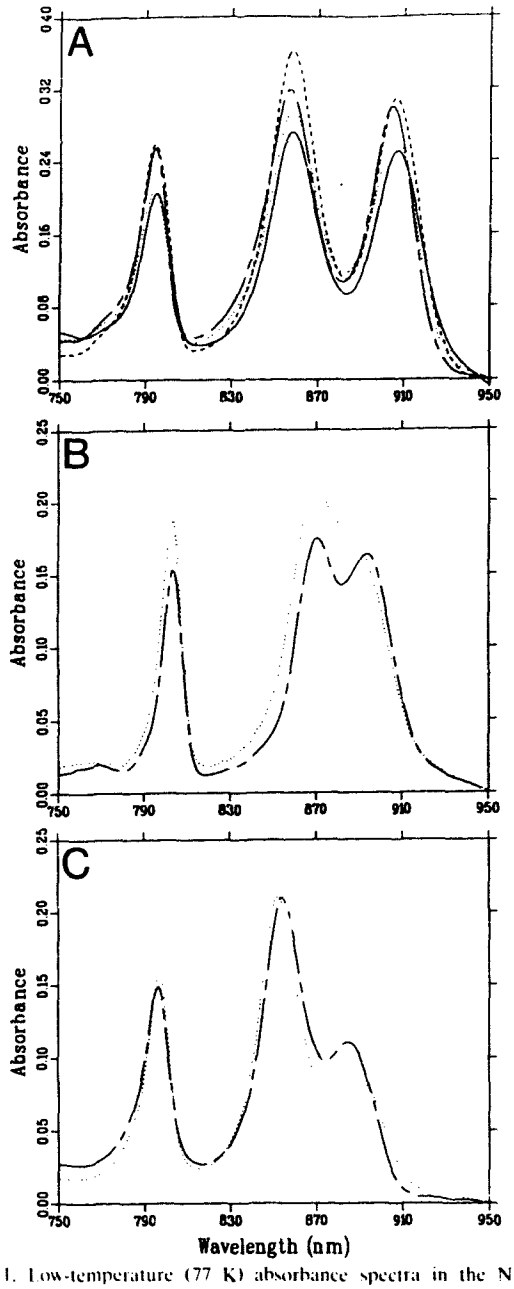

Fig. 1. Low-temperature (77 K) absorbance spectra in the NIR. Panel (A), *E. mobilis*; Panel (B), *Rps. palustris*; Panel (C), *Rb. sphaeroides*. Intact cells (———), chromatophores (- - - - -), $RC/LH_I/LH_{II}$ complexes isolated with octyl glucoside (·····) and $RC/LH_I/LH_{II}$ complexes isolated with cholate (— — —). Samples were prepared as described in Materials and Methods.

tested. This was comparable to the situation observed for *E. mobilis* (Fig. 1A) and *Rps. palustris* (Fig. 1B).

Fluorescence-emission spectra

Absorbance spectra measured at low temperature are indicative for changes in the arrangement of the pigments and/or proteins that may occur during the isolation of RC/antenna complexes from the photosynthetic membranes. To acquire information about the functional changes that might occur during this isolation, fluorescence spectroscopy was used. In order to study the energy transfer from B800 via B850 and B880 to the RC, emission spectra were recorded with excitation of the samples set at 800 nm. All samples were cooled in the dark to suppress RC fluorescence.

Fig. 2A shows the NIR fluorescence-emission spectra of cells, chromatophores and $RC/LH_I/LH_{II}$ complexes isolated from *E. mobilis*, taken at 77 K. Cells of *E. mobilis* displayed a fluorescence spectrum with a peak at 927 nm due to emission from LH_I. In chromatophores, weak additional fluorescence was observed around 890 nm, due to uncoupled LH_{II}. The 77 K fluorescence spectra of $RC/LH_I/LH_{II}$ complexes isolated from *E. mobilis* with either octyl glucoside or cholate (Fig. 2A) displayed two maxima, at 889 and around 922 nm, due to emission from LH_{II} and LH_I, respectively.

Similar spectra of $RC/LH_I/LH_{II}$ complexes from *Rps. palustris*, isolated with either octyl glucoside or cholate, are shown in Fig. 2B. In both spectra a maximum at 918 nm could be observed, due to LH_I fluorescence emission. In the spectrum of the complexes isolated with octyl glucoside, a weak LH_{II} emission was just visible, partly hidden in the blue wing of the LH_I emission peak.

In Fig. 2C the 77 K fluorescence-emission spectra of $RC/LH_I/LH_{II}$ complexes isolated from *Rb. sphaeroides* with either octyl glucoside or cholate are shown. Strikingly, in the complexes isolated with octyl glucoside, a very high fluorescence-emission peak at 912 nm, due to LH_I emission, could be observed. The LH_{II} emission was clearly visible as a shoulder at the blue side of the LH_I emission peak. In the spectrum of $RC/LH_I/LH_{II}$ complexes isolated with cholate, a much lower fluorescence was observed at 907 nm (due to LH_I emission). Compared to the complexes isolated with octyl glucoside, the fluorescence-emission of LH_I in complexes isolated with cholate, was 5 nm blue-shifted. The LH_{II} emission was barely visible in the complexes isolated with cholate, which is indicative of a tight coupling between LH_I and LH_{II}.

Fluorescence-excitation spectra

Fig. 3A presents the 77 K fluorescence-excitation spectra of different samples of *E. mobilis*. To avoid detection of emission due to detached LH_{II}, the fluorescence was recorded at 945 nm (or at 930 nm for the complexes isolated with octyl glucoside), which is in the red wing of the LH_I emission peak of *E. mobilis* (see Fig. 2A). The shape of the fluorescence-excitation spectra shown in Fig. 3A was in good agreement with the absorbance spectra observed at 77 K (Fig. 1A). In

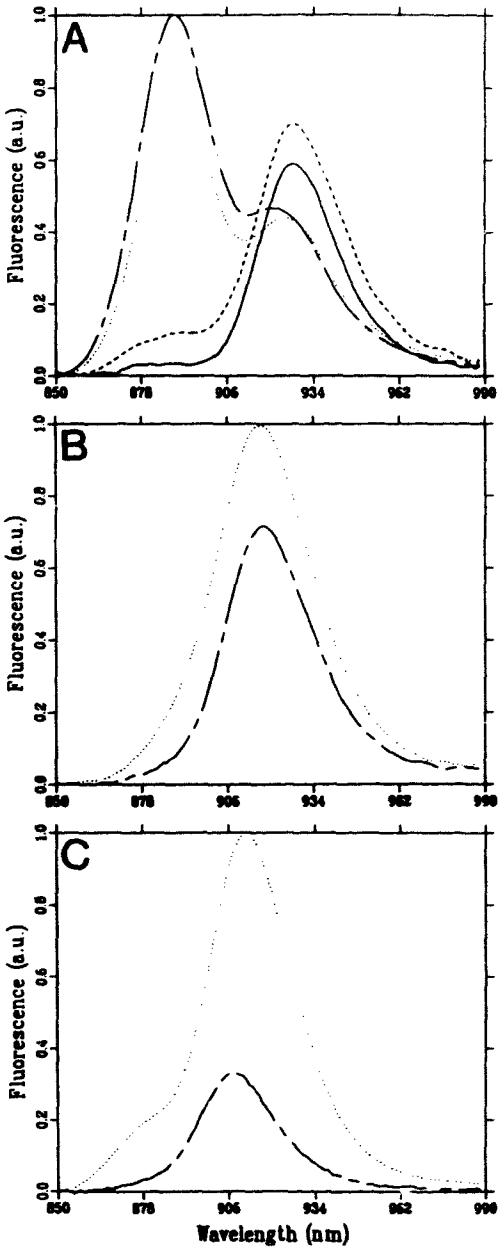

Fig. 2. Low-temperature (77 K) fluorescence-emission spectra in the NIR. Panel (A), *E. mobilis*; Panel (B), *Rps. palustris*; Panel (C), *Rb. sphaeroides*. Intact cells (———), chromatophores (- - - - - -), $RC/LH_I/LH_{II}$ complexes isolated with octyl glucoside (· · · · · ·) and $RC/LH_I/LH_{II}$ complexes isolated with cholate (— — —). Excitation wavelength 800 nm. The spectra were normalized on the basis of absorbance at 800 nm. Samples were prepared as described in Materials and Methods (a.u., arbitrary units).

the fluorescence-excitation spectra of $RC/LH_I/LH_{II}$ complexes isolated with cholate, the contribution of both B800 and B850 was decreased, compared to cells and $RC/LH_I/LH_{II}$ complexes isolated with octyl glucoside, indicating that the complexes isolated with cholate had a decreased efficiency of energy transfer between LH_{II} and LH_I. This decreased efficiency was consistent with the higher LH_{II} fluorescence emission of these complexes (Fig. 2A).

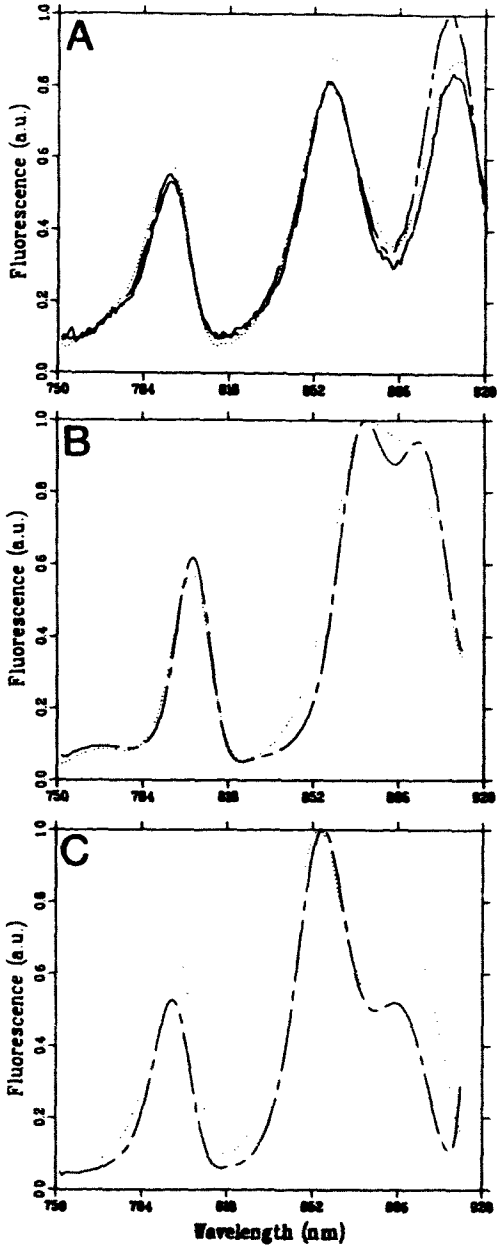

Fig. 3. Low-temperature (77 K) fluorescence-excitation spectra in the NIR. Panel A, *E. mobilis*, emission detected at 945 nm (except for octyl glucoside-$RC/LH_I/LH_{II}$ complexes, at 930 nm); Panel (B), *Rps. palustris*, emission detected at 930 nm; Panel (C), *Rb. sphaeroides*, emission detected at 930 nm. Intact cells (———), $RC/LH_I/LH_{II}$ complexes isolated with octyl glucoside (· · · · · ·) and $RC/LH_I/LH_{II}$ complexes isolated with cholate (— — —). Samples were prepared as described in Materials and Methods (a.u., arbitrary units).

In Fig. 3B the low-temperature NIR fluorescence-excitation spectra of $RC/LH_I/LH_{II}$ complexes isolated from *Rps. palustris* with either octyl glucoside or cholate, are shown. The fluorescence was detected at 930 nm, which was in the red wing of the LH_I emission maximum (see Fig. 2B). The fluorescence-excitation spectra in Fig. 3B closely resembled the corresponding absorbance spectra at 77 K (Fig. 1B) and indicated close to perfect excitation energy transfer from LH_{II} to LH_I in both preparations.

The low-temperature NIR fluorescence-excitation spectra of $RC/LH_I/LH_{II}$ complexes isolated from *Rb. sphaeroides* with either octyl glucoside or cholate, are shown in Fig. 3C. The fluorescence was detected at 930 nm. In complexes isolated with octyl glucoside, the relative contribution of LH_{II} was much lower, compared to $RC/LH_I/LH_{II}$ complexes isolated with cholate, indicating that, in addition to the disturbed energy transfer between LH_I and the RC (see Fig. 2C), the energy transfer between LH_{II} and LH_I also was strongly disturbed.

Energy transfer

Since the energy transfer between B800 and B850 is more than 95% efficient and unidirectional at 77 K [21,22], the fluorescence and absorbance data presented in the previous sections can be used to calculate the extent of energy transfer between LH_{II} and LH_I at 77 K. Since, in most investigated samples, the coupling between LH_I and the RC was intact, as judged by a low LH_I fluorescence-emission (see Fig. 2), the same data can be used as an illustration of the degree of energy transfer between the peripheral antenna and the RC. The only exception are the data presented for the $RC/LH_I/LH_{II}$ complexes isolated with octyl glucoside from *Rb. sphaeroides*, which showed a considerable disturbance of the energy transfer between LH_I and the RC, as judged by the high LH_I fluorescence-emission (see Fig. 2C).

In Table I the extent of energy transfer at 77 K between B850 and LH_I (and, therefore, between LH_{II} and the RC, see above) of different samples is presented. The relatively intense B850 fluorescence-emission observed for RC/antenna complexes from *E. mobilis* in Fig. 2A was due to an uncoupled fraction of LH_{II}, with a correspondingly high fluorescence yield. The spectra may be taken to suggest that the extent of energy transfer between LH_{II} and LH_I has remained almost unaltered (91%) in the $RC/LH_I/LH_{II}$ complexes isolated with octyl glucoside from *E. mobilis*. In $RC/LH_I/LH_{II}$ complexes isolated with cholate, a lower energy transfer of 75% was observed.

In the $RC/LH_I/LH_{II}$ complexes isolated from *Rps. palustris*, the extent of energy transfer between LH_{II} and LH_I was high (up to 93%) and nearly independent of the detergents used during isolation of the complexes (Table I).

TABLE I

Transfer efficiencies between B850 and LH_I in different samples from E. mobilis, Rps. palustris and Rb. sphaeroides

Efficiencies are estimated by comparison of the $B850/LH_I$ peak-height ratios in the fluorescence-excitation spectra (normalized on basis of the absorbance at 800 nm) and in the absorbance spectra (base-line corrected). The emission was detected at 945 nm for *E. mobilis* and at 930 nm for *Rps. palustris* and *Rb. sphaeroides*.

Samples	Transfer efficiency $B850 \rightarrow LH_I$ (%)
E. mobilis	
Cells	97
Chromatophores	95
Octyl-glucoside-$RC/LH_I/LH_{II}$	91
Cholate-$RC/LH_I/LH_{II}$	75
Rps. palustris	
Octyl-glucoside-$RC/LH_I/LH_{II}$	93 [a]
Cholate-$RC/LH_I/LH_{II}$	100
Rb. sphaeroides	
Octyl-glucoside-$RC/LH_I/LH_{II}$	63
Cholate-$RC/LH_I/LH_{II}$	94

[a] Due to overlapping absorption bands of B850 and RC/LH_I in these complexes (see Fig. 1B), the calculated efficiency may be less accurate.

The $RC/LH_I/LH_{II}$ complexes isolated with octyl glucoside from *Rb. sphaeroides* had a remarkably low energy transfer efficiency between LH_{II} and LH_I (63%). This corresponds to the pronounced shoulder in the emission spectrum upon 800 nm excitation (see Fig. 2C). The connection between LH_I and the RC has also been lost, as can be concluded from the high LH_I emission peak. All these effects were undone by replacing octyl glucoside for cholate, which yielded a functionally intact $RC/LH_I/LH_{II}$ unit.

CD spectra

CD spectra were measured to detect minor changes in the interactions between the chromophores that might have occurred during the isolation of RC/antenna complexes from *E. mobilis*.

The 77 K CD spectrum of RC/LH_I complexes isolated from *E. mobilis* (Fig. 4A) showed two biphasic signals with zero-crossings at 805 and 905 nm, respectively. The CD signal with a zero-crossing at 805 nm was due to the monomeric B800 bacteriochlorophyll in the RC, whereas the signal with a zero-crossing at 905 nm was, presumably, due to the BChl dimer in LH_I.

The 77 K CD spectrum of $RC/LH_I/LH_{II}$ complexes isolated from *E. mobilis* with octyl glucoside (Fig. 4B), showed several additional features. Besides the CD signals centred around 805 and 905 nm, due to RC and LH_I, two additional double CD bands from

LH_{II} with zero-crossings at, respectively, 796 and 865 nm, could be observed. In this spectrum, the ratio of the positive and negative signals due to RC and LH_I differed from the RC/LH_I complexes, but this is largely due to convolution of the CD bands in the $RC/LH_I/LH_{II}$ complexes. This convolution of narrow biphasic signals enhances changes in a CD spectrum reflecting only small changes in position and intensity of the individual bands. It also prevents a direct estimate of the absolute increase or decrease of individual bands.

These effects might also account for the small differences observed between the 77 K CD spectra of cells, chromatophores and cells exposed to 1% octyl glucoside for 30 min (Fig. 5). Compared to the CD spectrum of $RC/LH_I/LH_{II}$ complexes (Fig. 4B), in the CD spectrum of intact cells of *E. mobilis* (Fig. 5, upper trace) the negative CD signal, with a trough at 799 nm, was less pronounced. Furthermore, the zero-crossing of the LH_I CD signal was observed at 911 nm in cells, instead of 905 nm, as observed in the $RC/LH_I/LH_{II}$ complexes. Since both changes were also observed when cells of *E. mobilis* were exposed to 1% octyl glucoside for 30 min (Fig. 5, lower trace), these effects probably reflect changes that occurred when the complexes were solubilized by detergent. In chromatophores (Fig. 5, middle trace), an intermediate situation was observed for both the CD signal with a trough at 799 nm, as well as for the position of the zero-crossing of the LH_I CD signal. In chromatophores this zero-crossing was observed at 908 nm, instead of 911 nm, as observed in intact cells of *E. mobilis*.

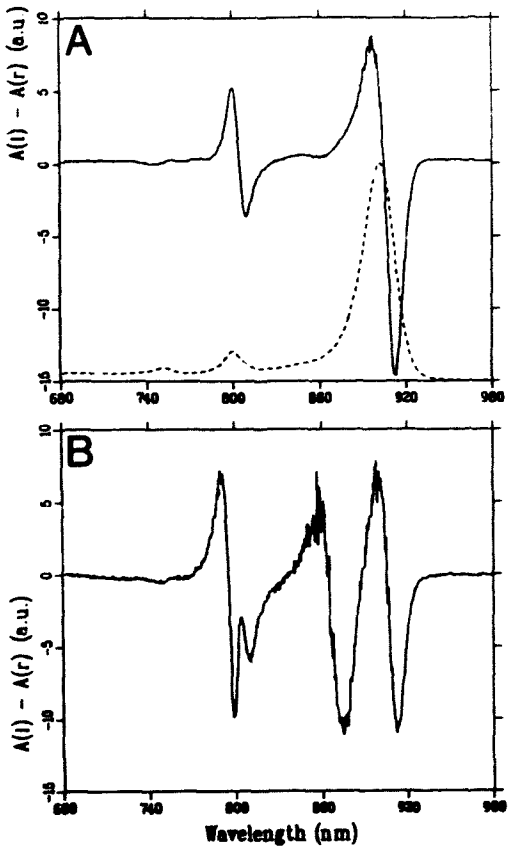

Fig. 4. Low-temperature (77 K) NIR absorbance and CD spectra of (A) RC/LH_I complexes and (B) $RC/LH_I/LH_{II}$ complexes isolated from *E. mobilis*. Absorbance spectrum (- - - - - -). CD spectra (———). Samples were prepared as described in Materials and Methods (the $RC/LH_I/LH_{II}$ complexes were isolated with octyl glucoside). The intensities of the CD spectra were normalized on the basis of $A_{(805)} = 1$. the error in the intensities is approx. 15% (a.u., arbitrary units).

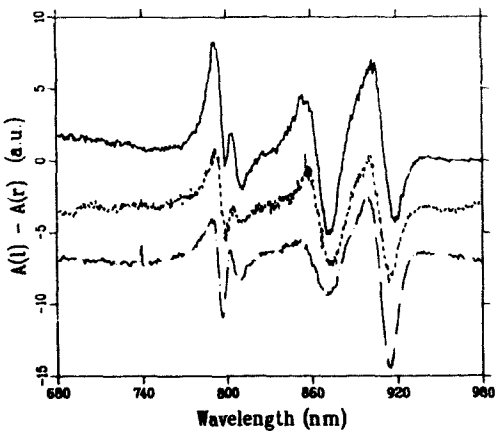

Fig. 5. Low-temperature (77 K) NIR CD spectra of cells and chromatophores of *E. mobilis*. Cells (———, upper trace), chromatophores (- - - - - -, middle trace), cells treated with 1% octyl glucoside (— · — · —, lower trace). Samples were prepared as described in Materials and Methods. The intensities of the CD spectra were normalized on basis of $A_{(805)} = 1$. the error in the intensities is approx. 15% (a.u., arbitrary units).

Discussion

Absorbance- and fluorescence spectra

In this paper the spectral properties at low temperature (77 K) of RC/antenna complexes from three purple bacteria *E. mobilis* BN 9903, *Rps. palustris* NCIB 8288 and *Rb. sphaeroides* 2.4.1 are described. By using the non-ionic detergent octyl glucoside, we are able to isolate $RC/LH_I/LH_{II}$ complexes from *E. mobilis* which are functionally intact as judged by both the 77 K absorbance spectrum and the energy transfer of up to 91% between the peripheral antenna complexes and the RC, as well as by the observed CD spectrum that in many details closely resembles the 77 K CD spectrum of intact cells. To isolate functionally intact RC/antenna complexes from *Rb. sphaeroides* and *Rps. palustris*, the ionic detergent cholate turns out to be a better detergent than octyl glucoside. In *Rb. sphaeroides*

octyl glucoside induces a blue-shift of the LH_I absorbance band and a decoupling between LH_{II} and LH_I and between LH_I and the RC. These data further underline that the interaction between the antenna complexes and the RC is different among the diverse bacteria. For the isolation of functionally intact RC/antenna complexes from different species, therefore, different detergents and solubilization conditions will have to be used. The results presented in Fig. 1 indicate that isolation with both types of detergent yields $RC/LH_I/LH_{II}$ complexes from *E. mobilis* with 77 K absorbance spectra comparable to those of intact cells and chromatophores. This in contrast to the situation observed for *Rps. palustris* and *Rb. sphaeroides*. In the RC/antenna complexes isolated with octyl glucoside from these latter organisms, noticeable blue- and red-shifts can be observed.

Distinct differences can be observed between the complexes isolated with different detergents when low-temperature fluorescence emission of the various samples is measured (Fig. 2). The $RC/LH_I/LH_{II}$ complexes isolated with octyl glucoside from *E. mobilis* display less LH_{II} fluorescence emission compared to the complexes isolated with cholate. In spite of the relatively intense LH_{II} emission in the octyl-glucoside-isolated complexes, compared to cells and chromatophores of *E. mobilis*, the functional uncoupling between LH_{II} and LH_I does not exceed 10% (Table I). The relatively high intensity of the LH_{II} fluorescence is due to the high intrinsic fluorescence of LH_{II} compared to LH_I [23]. The occurrence of some LH_{II} fluorescence in the (very gently) isolated chromatophores of *E. mobilis* (Fig. 2A) indicates that the functional association between the peripheral and the RC/core antenna complexes is easily disturbed in this organism.

The strong increase of LH_I and LH_{II} fluorescence in $RC/LH_I/LH_{II}$ complexes isolated from *Rb. sphaeroides* with octyl glucoside (Fig. 2C), shows that, during isolation of these complexes, the functional coupling between LH_{II} and LH_I as well as between LH_I and the RC, is disrupted. The red-shift of the fluorescence-emission peak of LH_I, observed in the complexes isolated with octyl glucoside (Fig. 2C), might be due to detergent-specific enlargement of the aggregation state of LH_I (Van Mourik, F. et al., unpublished results).

Functional interaction between LH_{II} and LH_I in Rps. palustris

From the low-temperature absorbance and fluorescence spectra of the different $RC/LH_I/LH_{II}$ complexes (Figs. 1–3), it can be concluded that the tightness of the association of the antenna complexes to the RC is stronger in *Rps. palustris* than in *Rb. sphaeroides* and *E. mobilis*. The functional coupling between the antenna complexes and the RC remains up to about 93% in the RC/antenna complexes from *Rps. palustris* isolated with octyl glucoside (see Table I). Addition of up to 0.5% of the ionic detergent sodium dodecyl sulphate to these complexes revealed no increase of LH_I or LH_{II} fluorescence before denaturation occurred (Leguijt, T. and Visschers, R.W., unpublished results), indicating that the coupling between the antenna complexes and the RC is very strong in this organism.

This conclusion contradicts earlier results reported by Molenaar et al. [14]. They concluded that the LH_{II} antenna from $RC/LH_I/LH_{II}$ complexes isolated from the same strain of *Rps. palustris*, did not significantly transfer excitation energy to the RC's. Additionally, the maximal oxidation rate of cytochrome *c* by the RC's of the RC/antenna complexes from *Rps. palustris* as described by Molenaar et al. [14], was only 10% of the oxidation rate we measured with $RC/LH_I/LH_{II}$ complexes from this organism (Leguijt, T. et al., unpublished results). The contrasting results might be due to prolonged storage of the chromatophores and the isolated RC/antenna complexes in liquid nitrogen by Molenaar et al. Preliminary experiments showed that the functional association between LH_{II} and LH_I as well as the maximal cytochrome *c* oxidation activity of our $RC/LH_I/LH_{II}$ complexes was disrupted after freezing of these complexes in liquid nitrogen.

CD spectra

Comparison of the 77 K CD spectra of intact cells, cells exposed to 1% octyl glucoside, chromatophores (see Fig. 5) and $RC/LH_I/LH_{II}$ complexes (Fig. 4B) of *E. mobilis*, clearly shows that they are very similar. No major changes in the interactions between the BChl *a* pigments can be detected, only some minor differences in the 800 nm region are visible. In this latter region, the CD signal from the B800 pigments of LH_{II} is superimposed on the CD signal from the B800 monomeric pigments of the RC.

The 77 K CD spectrum of RC/LH_I complexes from *E. mobilis* is comparable to room-temperature spectra of RC/LH_I complexes isolated from *Rps. palustris* and *Chromatium vinosum* [24,25]. The absorbance maxima of the antenna complexes and the RC are, however, red-shifted in our experiments due to sharpening of the absorption bands at 77 K. The double CD band with a zero-crossing at 905 nm is presumably due to the BChl dimer of LH_I, comparable to the signal observed in RC/LH_I complexes from *Rps. palustris* and *Chr. vinosum* [24,26] and the B890 antenna from *Rhodospirillum rubrum* [27], although it is also possible that the CD signal of the RC is superimposed on the CD signal of the B890-BChl's, as reported for *Rps. palustris* [25].

Since the intensity of the CD signals from the B850 and B890 pigments appear to be affected by detergent treatment, it would be interesting to investigate whether

these changes can be reversed after dialysis of the detergent and incorporation of the complexes into liposomes.

The data presented in this paper demonstrate that, for the isolation of functionally intact RC/antenna complexes, different detergents have to be used, since the tightness of the interaction between the antenna complexes and the RC is different among the investigated organisms. With the procedure described here, we are able to isolate functionally intact RC/antenna complexes from three well-characterized photosynthetic purple bacteria: *E. mobilis* BN 9903, *Rps. palustris* NCIB 8288 and *Rb. sphaeroides* 2.4.1. As mentioned in the Introduction, *Ectothiorhodospiraceae* grow under extreme saline and alkaline conditions. We did not find adaptations however, in either the structural (cf. Ref. 16), or the functional, properties of the RC/antenna complexes of *E. mobilis*, that can be directly related to these extreme growth conditions.

This study introduces the possibility of reconstituting the isolated RC/antenna complexes from *E. mobilis* into liposomes, which gives us the opportunity of obtaining a more detailed insight in the photosynthetic and bioenergetic machinery of the *Ectothiorhodospiraceae*.

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