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Spectral and kinetic effects accompanying the assembly of core complexes of *Rhodobacter sphaeroides*

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ABSTRACT

In the present work, spectral and kinetic changes accompanying the assembly of the light-harvesting 1 (LH1) complex with the reaction center (RC) complex into monomeric RC-LH1 and dimeric RC-LH1-PufX core complexes of the photosynthetic purple bacterium *Rhodobacter sphaeroides* are systematically studied over the temperature range of 4.5–300 K. The samples were interrogated with a combination of optical absorption, hole burning, fluorescence excitation, steady state and picosecond time resolved fluorescence spectroscopy. Fair additivity of the LH1 and RC absorption spectra suggests rather weak electronic coupling between them. A low-energy tail revealed at cryogenic temperatures in the absorption spectra of both monomeric and dimeric core complexes is proved to be due to the special pair of the RC. At selected excitation intensity and temperature, the fluorescence decay time of core complexes is shown to be a function of multiple factors, most importantly of the presence/absence of RCs, the supramolecular architecture (monomeric or dimeric) of the complexes, and whether the complexes were studied in a native membrane environment or in a detergent - purified state.

1. Introduction

Photosynthesis begins with the absorption of solar photons by the densely packed pigment chromophores in the light harvesting (LH) protein complexes. The electronic excitation energy of a collection of pigments (exciton) eventually migrates to the reaction centre (RC), where a photochemical charge separation takes place [1–3]. The purple phototrophic bacterium *Rhodobacter* (*Rba.*) *sphaeroides* provides a valuable model system for the study of the photosynthetic exciton energy transfer and trapping, due to the availability of high-resolution structures of the major LH and RC proteins, and a detailed knowledge of the biogenesis and organisation of their photosynthetic membranes (see [4] for a recent review). In *Rba. sphaeroides*, the photosynthesis apparatus is arranged into an intracytoplasmic membrane system commonly called chromatophores [5,6]. The chromatophores are composed of two main types of transmembrane protein complexes: the core complex comprised of a light harvesting complex 1 (LH1) encircling a RC and a peripheral or distal light harvesting complex 2 (LH2). In both LH1 and LH2 the basic building block for in vivo assembly is a heterodimer of membrane-spanning α-helical α- and β-polypeptides, with each apoprotein noncovalently binding three (LH2) or two (LH1) bacteriochlorophyll *a* (BChl) molecules. While the large scale architecture of LH2, a closed planar ring of apoproteins in the membrane plane, is relatively constant, the core complex design strongly varies from species to species. In wild type *Rba. sphaeroides*, for example, the presence of additional PufX polypeptide cuts the apoprotein ring and leads most of the core complexes to assemble into a nonplanar S-shaped array of 28 αβ-$BChl_2$ structural units encircling two RCs [5,7]. This is the so-called dimeric core complex shortly indicated here as RC-LH1-X. Absence of PufX in certain native [8,9] and mutant [10] complexes results in 16 αβ-$BChl_2$ structural elements of LH1 fully encircling a single RC, the planar monomeric core complex RC-LH1.

Whilst our understanding of spectroscopic features of the detergent isolated (further distinguished by *i*, iLH1 etc.) elements of the bacterial photosynthetic unit such as LH2, LH1, and RC pigment-protein complexes is extensive, there is still much we do not fully comprehend when the complexes organize into functional membrane networks (further distinguished by *m*) [11–19]. Therefore, in the present work, we systematically study and interpret the multiple factors that determine the spectral and kinetic parameters of different core complexes from *Rba. sphaeroides* upon assembly into functional core complexes.

Abbreviations: BChl, bacteriochlorophyll *a*; LH, light-harvesting; iLH, isolated LH complex; mLH, membrane LH complex; RC, reaction center; SDF, site/state distribution function; X, PufX polypeptide.

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2. Materials and methods

2.1. Sample preparation

The samples were prepared as described earlier [10,20–22]. Shortly, the DD13 double deletion strain of *Rba. sphaeroides* [10] was complemented with the desired genes to produce photosystems containing only the RC, only the LH1 or only the monomeric RC-LH1 complexes. The RC-LH1-X complexes were extracted and purified from the wild-type *Rba. sphaeroides* strain 2.4.1 that has been genetically manipulated by deleting the crtC of the native carotenoid biosynthetic pathway. This strain develops core complexes mainly in dimeric RC-LH1-X form, irrespective whether the cells were grown photosynthetically or semiaerobically [22]. The membrane samples with RC-LH1-X used here were also from this strain. Complete deletion of crtC yield neurosporene as the major carotenoid. As a result the type of carotenoid present in the dimeric core RC-LH1-X materials (neurosporene) was different from those in the LH1-only and monomeric core RC-LH1 materials (mainly spheroidenone). The samples were diluted with 10 mM TRIS HCl (pH 7.9) or 20 mM HEPES (pH 7.8) buffer before use. A specific detergent was added to the isolated complexes to prevent aggregation: 3 mM DHPC in case of LH1 and RC-LH1, and 0.03% β-DDM in case of RC-LH1-X. Addition of glycerol to the buffer with a 2:1 volume ratio secured transparent glassy samples for low temperature measurements. The absorption and fluorescence spectra, as well as the kinetics checked at ambient temperature of the samples with and without glycerol were similar. The samples were stored at −78 °C in a deep freezer.

2.2. Spectroscopy

The steady state absorption and fluorescence spectra were recorded using a 0.3 m spectrograph (Shamrock SR-303i, Andor Technology) equipped with a thermo-electrically cooled CCD camera (DV420A-OE, Andor Technology). The fluorescence spectra, corrected for sensitivity of the experimental set-up, were excited at 407 nm using a diode laser. For absorption measurements, a high-stability tungsten light source (BPS100, BWTek) was employed. If not indicated otherwise, the spectral resolution of the measurements was 0.4 nm. Spectral hole-burning and resonant fluorescence excitation was performed using a Ti: sapphire laser (model 3900S, Spectra Physics) of 0.5 cm^{-1} linewidth pumped by a Millennia Prime solid state laser (Spectra Physics). The zero-phonon holes were burned across the long-wavelength shoulder of the inhomogeneously broadened B875 absorption band with constant burn fluence of 0.8 J/cm^2.

In fluorescence kinetics measurements the samples were excited at 860 nm with ~100 fs pulses of femtosecond mode-locked Ti:sapphire laser (Coherent Mira-900) at 76 MHz repetition rate. The excitation intensity and the pulse repetition rate was used such that practically all the RCs in core complexes were in the photo-chemically inactive (special pair photo-oxidized) state. At the same time, the excitation conditions were deliberately selected to avoided exciton annihilation effects, either of singlet-triplet or singlet-singlet type, see [23–25]. The fluorescence decay was recorded with a time-correlated single photon counting system (SPC-150, Becker & Hickl GmbH) using a hybrid photomultiplier detector (HPM-100-50, Becker & Hickl GmbH) with temporal resolution of ~140 ps. When better temporal resolution was required, a synchroscan streak camera (Hamamatsu C1587) with temporal resolution of ~10 ps was employed. To select the proper wavelength of detected emission the double subtractive dispersion monochromator (DTMc300, Bentham Ltd.) was applied. The spectral resolution used in time-resolved measurements varied between 2 and 4 nm.

Plastic (PMMA) cuvettes of 10 mm path length and gelatine capsules of 4 mm diameter were used as sample containers for absorption and fluorescence measurements, respectively. In the latter case, an optical density ≤0.05 in the measuring volume was used to avoid emission reabsorption effects. To control the sample temperature, either a liquid nitrogen cryostat (Optistat DN, Oxford Instruments) or a liquid helium cryostat (Utreks) was used. The temperature was controlled with the precision of ±0.5 K.

3. Results and discussion

3.1. Overview optical absorption and steady state emission spectra of core complexes

Shown in Fig. 1 are the absorption and fluorescence spectra of the native photosynthetic membrane bound LH1 complexes devoid of the RC complex (mLH1) and the monomeric RC-LH1 core complexes (mRC-LH1) measured between 500 and 1000 nm, and at temperatures 4.5 K and 295 K. In both these samples the LH1 complex contains 16 αβ-$BChl_2$ structural elements and forms a closed ring, allowing their direct comparison with respect to the presence of the RC complex.

In case of the LH1 complex the dominant absorption band peaks at 877 nm at ambient temperature, see Fig. 1a. This band denoted as B875 is related to the lowest singlet electronic transition of a ring of tightly organized BChl pigment chromophores in the protein complex [8,9]. A strong exciton coupling within and between the dimeric structural elements is considered to be one of the main reasons of the large spectral shift of the B875 band as compared to the singlet (Q_y) absorption spectrum of individual BChl pigments [26–31]. Although the exciton origin of the B875 spectrum well explains its significant redshift and narrowing with temperature [32–35], a fraction of the shift may also be due to the mixing of the exciton states with charge transfer states, as reflected by the strong Stark effect observed in LH1 and LH2 complexes [36–38]. The weak absorption band at 589 nm corresponds to the Q_x electronic transitions of the BChl chromophores [39]. Collective effects related to this feature are feebler than those for the B875 band due to smaller oscillator strength of the Q_x transition compared with the Q_y transition as well as to different geometry of the transition dipole moments related to these bands. The broad spectral background toward short wavelengths, revealing a structure at low temperatures, is associated with the optically allowed S_2 vibronic state manifold of

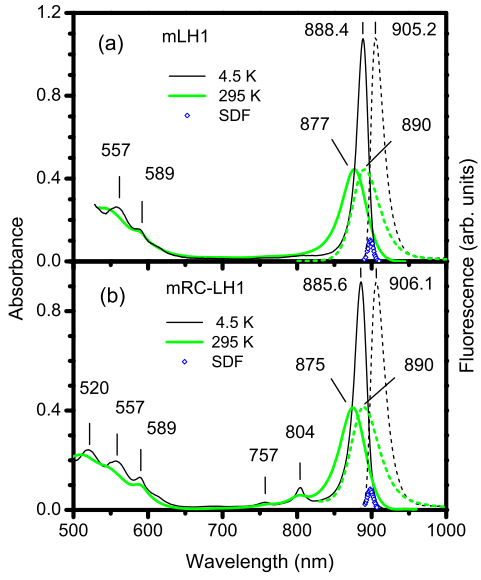

Fig. 1. The area-normalized absorption (solid curve) and fluorescence (dashed curve, excited at 407 nm) spectra of LH1 (a) and RC-LH1 (b) membrane complexes from *Rba sphaeroides* recorded at 4.5 K (black) and 295 K (green). Shown with blue diamonds is the distribution of the lowest energy excitonic states, SDF. Numbers indicate spectral positions in nanometers.

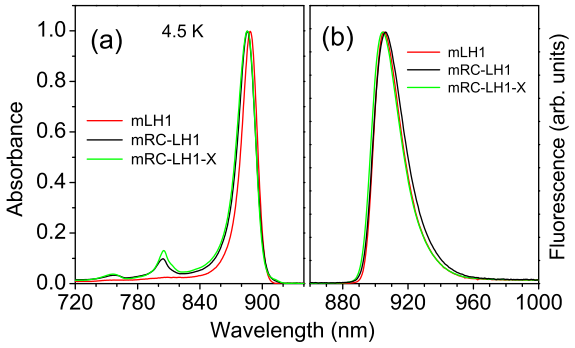

Fig. 2. Comparison of the peak-normalized absorption (a) and fluorescence (b) (excited at 407 nm) spectra of the membrane-embedded monomeric and dimeric core complexes from *Rba. sphaeroides*. The spectra were measured at 4.5 K.

carotenoid chromophores also present in the LH1 complex (mainly spheroidenone in the pictured case).

Formation in the membrane of core complexes complete with LH1 and RC (mRC-LH1) results in subtle but characteristic changes of the absorption spectrum (Fig. 1b). Most notable are the two new bands at 804 nm and 757 nm associated with the absorption of pigment cofactors in the RC complex, two accessory BChls and two bacteriopheophytins, respectively. The 589-nm band now accounts for the BChl pigment chromophores from both LH1 and RC complexes. Similarly, the carotenoid pigments present in both the LH1 and RC structures collectively contribute into the spectrum below 640 nm. The Q_x electronic transitions of bacteriopheophytin chromophores in RC donate to this spectrum around 530 nm.

Upon lowering the temperature the spectra consistently redshift and become narrower, hence acquiring a more clear-cut structure. Shown also in Fig. 1 with diamonds are the inhomogeneous distributions of the lowest energy exciton polaron states (SDF) determined by hole-burning action spectroscopy at 4.5 K [40,41]. We will return to these more subtle spectral aspects shortly.

Fig. 2a compares the B875 peak-normalized absorption spectra of the membranes containing monomeric (mRC-LH1) and dimeric (mRC-LH1-X) core complexes at 4.5 K with each other and with that of the membranes containing sole LH1 complexes (mLH1). One can notice that all the core complexes show very similar spectra, notwithstanding the presence or absence of PufX. A blue shift by 1–2 nm has been modeled for the exciton absorption spectra in the samples with PufX due to breaking the complete ring of the coupled pigments [28,42,43], which the present data are not able to confirm within the experimental uncertainty. However, compared with mLH1, the absorption spectra of core complexes complete with RC appear systematically bluer as well as broader; see more complete account of data in Table 1.

The fluorescence emission spectra of the core complexes related to the B875 exciton polarons show a single relatively broad band (Figs. 1b and 2b). All the spectra appear similar, which is rather expected, in view that they are associated with just the LH1 excitons, see below.

Table 1 compares major spectral data for the membrane-protected and detergent-isolated core complexes studied at ambient temperature and at 4.5 K. While the spectra at room temperature practically coincide, at cryogenic temperatures, the spectra related to the membrane complexes appear slightly but systematically redder than the spectra of isolated complexes (by 1.1 to 2.3 nm in absorption and by 2.9 to 3.7 nm in emission for different pairs of samples). In accordance with [13,44] the larger red shift observed in the emission of membrane systems can be assigned to the inter-complex excitation energy transfer available in the connected network of spectrally disordered membrane antenna complexes and not in the solid solution of isolated complexes.

In agreement with the previously published data on isolated LH1 complexes [29,35,36], the SDF distributions peak around 895–898 nm. The width of the SDF recorded for isolated complexes is broader than that for membrane complexes, consistent with their conceivably greater heterogeneity. As compared to monomeric core complexes, dimeric core complexes do not appear to contribute any additional spectral heterogeneity. This suggests that nonplanar dimeric core structures are nearly as rigid as planar monomeric core complexes.

3.2. Detailed revision of the absorption line shape of core complexes revealing the contribution of the special pair of RC

In relation with plant photosystems, discussion about the so called antenna red states is ongoing [46–51]. Therefore, we carried out a detailed study of the red tails of the low-temperature absorption spectra of core complexes with and without the RC.

As demonstrated in Fig. 3a, these spectra reveal systematic deviations - the tails of the core complexes complete with RC extend significantly further toward the red than that of the sole LH1 complex. Furthermore, while the absorption tail of the sole LH1 complex closely matches the SDF distribution [45], distinct difference is observed in the case of core complexes with the RC in place. Fig. 3b illustrates this notion in case of the mRC-LH1 sample by showing that past about 905 nm the absorption spectrum and the SDF gradually deviate from each other toward longer wavelengths. It is worth noticing that the SDF is related just to the LH1 component of the core complex (either mRC-LH1 or mRC-LH1-X), because burning holes in RC is a relatively very inefficient process [37,38].

Low absorbance severely limits the accuracy of the action spectroscopy at long wavelengths. To push the line shape analysis further toward longer wavelengths, a fluorescence excitation method was used, as demonstrated in the inset of Fig. 3b. There is no doubt, the fluorescence intensity indicated with green diamonds and following the SDF profile clearly deviates from the absorption spectrum in the long-wavelength tail region. This proves that the red tail present in the low-temperature absorption spectrum of bacterial core complexes is associated with their RC rather than LH component, because no such feature is observed in sole antenna (LH1 or LH2) complexes. It is

Table 1
Spectral characteristics of the core complexes from *Rba. sphaeroides*.[a]

	Absorption peak (nm)		SDF at 4.5 K		Fluorescence peak (nm)	
Sample	295 K	4.5 K	Peak (nm)	Width[b] (cm^{-1})	295 K	4.5 K
iLH1[c]	876.7 ± 0.6	886.1 ± 0.4	896.0 ± 0.4	118 ± 8	892.6 ± 0.6	901.5 ± 0.4
mLH1	876.8 ± 0.6	888.4 ± 0.4	898.4 ± 0.4	108 ± 8	890.4 ± 0.6	905.2 ± 0.4
iRC-LH1	874.9 ± 0.6	884.5 ± 0.4	N/A[d]	N/A[d]	887.8 ± 0.6	902.5 ± 0.6
mRC-LH1	874.8 ± 0.6	885.6 ± 0.4	898.0 ± 0.4	120 ± 8	889.8 ± 0.6	906.1 ± 0.4
iRC-LH1-X	873.4 ± 0.6	884.0 ± 0.4	894.6 ± 0.4	128 ± 8	890.3 ± 0.6	901.8 ± 0.4
mRC-LH1-X	875.6 ± 0.6	885.6 ± 0.4	897.6 ± 0.4	118 ± 10	N/A[d]	904.7 ± 0.4

[a] Shown with ± is the standard deviation according to multiple independent measurements.
[b] Determined as the full width at half maximum.
[c] Data reproduced from [45].
[d] N/A-Data not available.

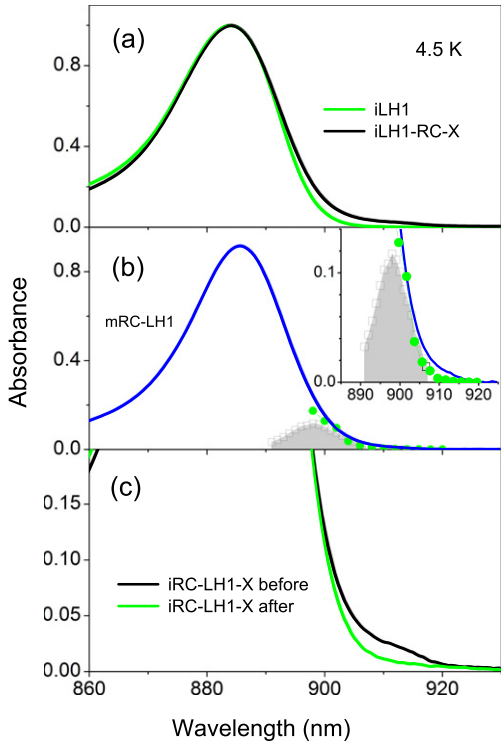

Fig. 3. (a) Overlapping absorption spectra of isolated LH1 (green line) and dimeric core RC-LH1-X (black line) complexes. For better comparison the spectra were normalized with respect to the B875 absorption peak and the spectrum of the LH1 complex was shifted toward shorter wavelengths by 2.0 nm. (b) Hole burning action (open squares and grey area, the same as in Fig. 1b) and fluorescence excitation (green diamonds) spectra of monomeric core complex mRC-LH1 scaled arbitrarily to match the absorption spectrum (blue line). The inset shows amplified tail region between 885 and 925 nm. (c) Amplified absorption spectra of the iRC-LH1-X complex before (black line) and after (green line) bleaching with a laser light of 50 mW/cm^2 intensity at 532 nm when cooling down from 250 to 120 K in about 10 min. All spectra were recorded at 4.5 K.

similarly obvious that the low-temperature emission of core complexes is generally due to LH1 rather than RC, despite the RC state being energetically the lowest. This is because, as already remind before, at the used excitation intensity the RC emission is effectively quenched by photo-oxidation of the RC [23,52].

Fig. 4 compares the absorption spectra of the membrane RC-LH1 and RC complexes at ambient and cryogenic temperatures. It is clear

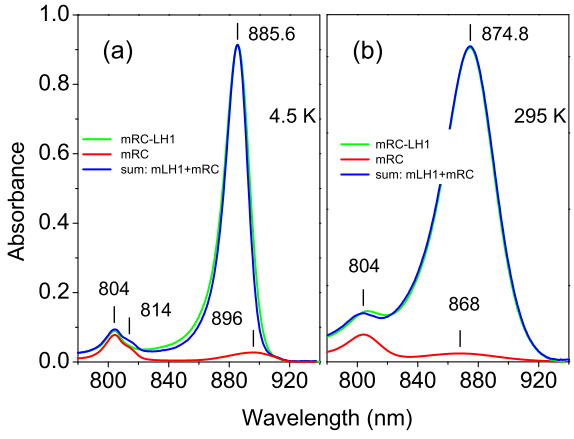

Fig. 4. Comparison of the absorption spectra of membrane RC-LH1 complexes (green line) at 4.5 K (a) and 295 K (b) with the properly scaled sum spectrum (blue line) of membrane RC (red line) and LH1 (not shown) complexes. For the match the LH1 spectrum was shifted toward blue by 2.8 nm at 4.5 K and by 2.2 nm at 295 K.

that the only RC band that significantly overlaps with the B875 band of LH1 is related to the absorption of the primary electron donor, a pair (commonly named special pair) of BChl molecules. This band peaks at 896 nm at 4.5 K and at 868 nm at ambient temperature.

The special pair gets readily photo-oxidized upon illumination with visible light, which results in bleaching out of the special pair band from the absorption spectrum of RC (see [53] for a review). Significant loss of the red tail absorption, as shown in Fig. 3c, upon illumination of the core complex during the sample cooling thus uniquely validates the involvement of the special pair in the formation of the absorption red tail at low temperatures. The estimated special pair/B875 integral absorption ratio in Fig. 4a is 1:20, slightly less than expected from the involved pigment ratio (1:16). This deficiency is due to partial oxidation of the special pair by stray light during the measurements. We deliberately avoided using external reductants in these trials, not knowing how they might influence the low-temperature data.

One can further notice from the fits presented in Fig. 4 that at 4.5 K the special pair band (peak at 896 nm) is much redder as compared the B875 band of LH1 (~886 nm). The situation at room temperature is reversed, as the special pair band (868 nm) occurs bluer than the B875 band (~875 nm). The unusually high thermal sensitivity of the special pair absorption band position is well known from the studies of isolated RC complexes [53], being related to the charge transfer origin of this band [54]. Here we first show that the vastly different thermal shift rates of the special pair [53] and B875 [53] bands directly influences the interpretation of the spectra of core complexes containing RC. The nature of the long-wavelength absorption spectrum tail of these complexes changes with temperature from being of the LH1 origin at around ambient temperatures to that of the RC origin at cryogenic temperatures.

An evidence for the exciton interactions between the RC and LH1 complexes was obtained from a comparison of absorbance-detected magnetic resonance triplet-minus-singlet spectra of chromatophores and isolated RCs of *Rba. sphaeroides* [55]. This is in contrast with the present data demonstrating an almost perfect overlap of the absorption spectrum of the self-assembled monomeric core complex (mRC-LH1) and the sum of the absorption spectra of the native membrane-embedded LH1 and RC complexes all over the temperature range from 4.5 K to room temperature (Fig. 4). Such a close correspondence can only be explained by relatively weak coupling between the RC and LH1 exciton states, also confirmed by some calculations [56]. Despite the weak coupling the RC has a principal role in functioning of core complexes, as will be discussed in subsequent sections.

3.3. Spectrally selective decay of exciton polarons in the core complexes at 5 K

Spectrally dispersive emission kinetics was previously observed in both isolated and membrane LH2 complexes at low temperatures [13,41,57,58]. Here we report about similar measurements conducted at cryogenic temperatures on core complexes of various complexities, starting from sole LH1 complexes devoid of RC either in isolated or membrane-embedded state (results presented in Fig. 5) and continuing with monomeric and dimeric core complexes complete with RC (Fig. 6).

As can be seen in Fig. 5a, in the absence of the RC, the dependences for the isolated LH1 complexes and for the complexes embedded in native membranes (the latter allowing excitation energy transfer between the complexes) are totally different. The isolated LH1 complex shows a rather long fluorescence lifetime of ~1.4 ns at the blue edge of the emission spectrum, which gradually shortens toward longer wavelengths until stabilizing at the level of about 1 ns around 895 nm, close to the maximum of the SDF. In the membrane, in contrast, the blue-edge lifetime of the emission spectrum becomes so short that it reaches temporal resolution of our set up. Toward longer wavelengths the lifetime gradually elongates until past ~915 nm it stabilizes at the same level (~1 ns) as in the isolated LH1 complex. We further notice that if

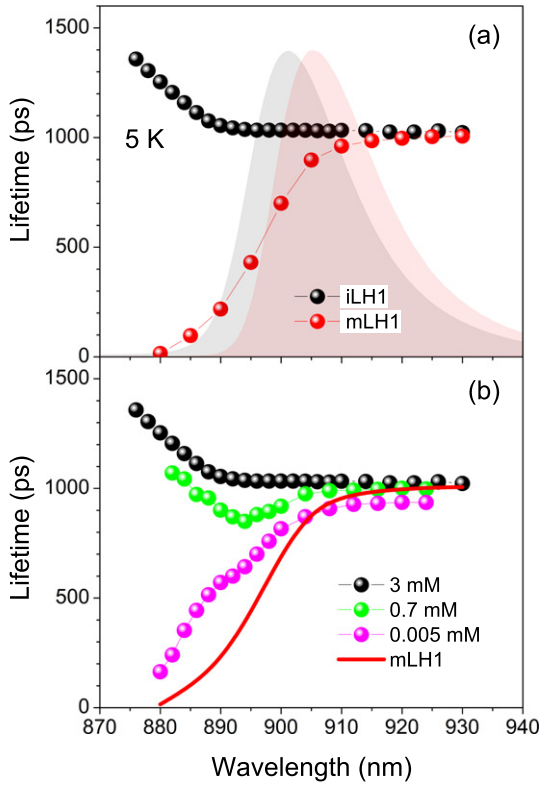

Fig. 5. Fluorescence lifetime in isolated and membrane LH1 complexes measured at 5 K in dependence of the recording wavelength. (a) Data for isolated (black, with 3 mM detergent added) and native membrane-embedded (red) LH1 complexes. Reference fluorescence spectra of the samples using similar color codes are shown in the background. (b) Data for purified complexes with different detergent concentrations indicated. Reference membrane data is drawn by red line. The weighted lifetime is presented for membrane and aggregated complexes. In all cases the fluorescence was excited at 860 nm and recorded with spectral resolution of 2 nm. The lines connecting data points are for leading the eye only. See text for further explanations.

in isolated complexes the decay is mono-exponential, it is generally double-exponential in the membranes, turning to mono-exponential only past 915 nm. For multi-exponential kinetics an average (weighted) lifetime is presented as follows: $\tau = \sum_i A_i\tau_i / z\sum_i A_i$, where A_i and τ_i are the exponential component amplitude and decay time, respectively.

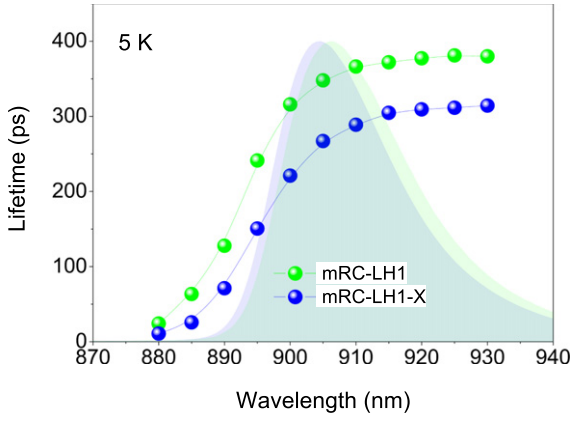

Fig. 6. Fluorescence lifetime as a function of recording wavelength at 5 K for the membrane-embedded monomeric (green) and dimeric (blue) core complexes in the presence of RC. The fluorescence was excited at 860 nm and recorded with spectral resolution of 4 nm. Saturating excitation intensity was used corresponding to closed RC state (most RCs photo-oxidized). Reference fluorescence spectra of the samples are drawn using corresponding color codes. The lines connecting data points are for leading the eye only.

The effect of connectivity is further demonstrated in Fig. 5b, where the kinetic data for purified LH1 complexes with different concentration of added detergent are shown. The sample with the highest concentration (3 mM) of detergent represents fully isolated complexes, while that with the lowest concentration (0.005 mM) corresponds to mostly aggregated complexes. The rest of the samples studied contained intermediate concentrations (0.7, 0.2 and 0.02 mM) of detergent. In accordance with one's expectation, the primary effect of the increasing connectivity of the LH1 complexes (which presumably follows the decreasing detergent concentration) is the acceleration of the short-wavelength kinetics. The kinetic traces related to the samples of different connectivity cover almost the whole space between the limits set by isolated and membrane complexes (only a few of them are shown in Fig. 5b for clarity). The greater aggregation is accompanied by a red-shift of the fluorescence spectra (data not shown). A fraction of purified LH1 complexes resists aggregation even at very low detergent concentration. This is reflected by specific shape of the weighted lifetime data and also by spectral dependence of the longest lifetime component, which in case of all concentrations follows that of isolated complexes, though with changing amplitude (data not shown).

In the literature, there are earlier dispersive fluorescence lifetime data on isolated LH1 complexes [40,41]. These data, if compared with the present data reveal signs of aggregation. Despite apparently similar sample preparation procedures used in both studies the set of data in Fig. 8b of Ref. [40,41] reminds that in Fig. 5b with a smaller, 0.7 mM detergent concentration, rather than that with a 3 mM concentration. The origin of this discrepancy is unclear, but the fact itself once again highlights the need to pay special attention to the sample preparation conditions in this type of detailed work.

Comparable dramatically different dispersive emission kinetics in case of individual (isolated) and connected (membrane) light-harvesting complexes was previously observed for LH2 proteins [13,41,57,58]. The specific kinetics with longer lifetime at shorter recording wavelengths in case of isolated complexes was assigned to a distribution of fundamental properties of exciton polarons in an energetically heterogeneous ensemble of individual complexes [56]. The lifetime distribution characteristic for individual complexes turns out to be completely veiled in an energetically connected membrane networks due to excitation energy transfer and funneling toward energetically lowest complexes [59–61].

The steep dispersive fluorescence kinetics, which were observed in the core membranes lacking RCs is also qualitatively followed in the core membranes that include RCs, see Fig. 6. In all cases the faster lifetimes are recorded at shorter wavelengths and slower ones at longer wavelengths. However, quantitative data are rather different. This is most clearly seen when the limiting long-wavelength (past 915 nm) lifetimes are compared. In the RC-less mLH1 sample this lifetime is ~1 ns, while in the membrane-embedded core complexes complete with RCs it is 300–400 ps, more than twice shorter.

An evident cause for that significant shortening of the core exciton polaron lifetime is additional quenching by RCs. In photosynthetic bacteria even closed RC (as in present case) effectively deactivate antenna excitons [52]. Furthermore, a much longer lifetime has been observed in case of monomeric core complexes than in dimeric core complexes (~380 vs. 310 ps in mRC-LH1 and mRC-LH1-X, respectively). This is consistent with the recently discovered higher quenching efficiency of dimeric core complexes in chromatophores of a specially designed mutant species of *Rba. sphaeroides* [23]. Quantitative modeling of the monomeric-dimeric core quenching effect is underway in our laboratory, being the subject of a forthcoming publication.

3.4. Temperature dependence of the fluorescence decay

Fig. 7a depicts the temperature dependence of the fluorescence decay time for the studied complexes in the temperature range of 5–300 K. In agreement with a previous report [33], the decay time of

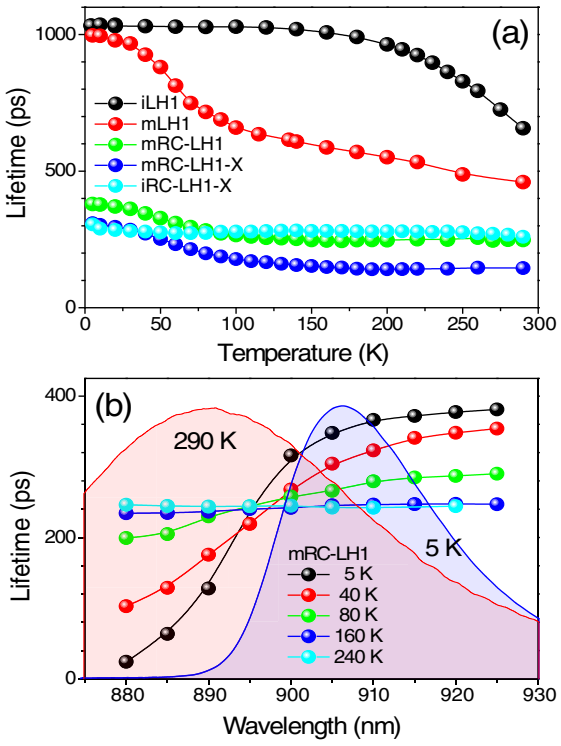

Fig. 7. Fluorescence decay time as a function of temperature (a) in various indicated core complexes and as a function of recording wavelength in membrane RC-LH1 complexes (b). The fluorescence was excited at 860 nm and recorded at red side of the fluorescence spectrum (at 920 nm between 5 and 230 K and at 890 nm above 230 K) with spectral resolution of 4 nm. In panel (b) the reference fluorescence spectra at 5 K (blue) and 290 K (red) are given. The lines connecting data points are for leading the eye only.

isolated LH1 complexes stays practically constant at low temperatures up until ~120 K and then gradually declines, reaching a value of ~600 ps at ambient temperatures. The isolated core complexes complete with the RC behave similarly, except at lower lifetime values. For example, the iRC-LH1-X sample keeps a nearly constant, ~300 ps, lifetime to almost 230 K, dropping only marginally afterwards. In the membrane samples, in contrast, the lifetime begins to decline already at very low temperatures and the drop continues all the way up to ambient temperatures, where the lifetime value reaches about half of its initial value at 5 K.

It is clear that the fluorescence quenching mechanisms dominating in isolated and membrane complexes must be different. The quenching observed in isolated complexes is due to inherent thermally activated non-radiative processes in the B875 assembly of BChl chromophores. The present data, like previous data on LH2 complexes [13,41,57,58], indicate that this is a relatively slow process. The generally faster quenching observed in membrane systems can be understood within the already described paradigm of inter-connected network of LH and RC complexes, where the occasional quenchers localized in separate membrane areas become increasingly available for quenching with the raising temperature.

The steep change of the light-harvesting exciton polaron lifetimes over the fluorescence band was demonstrated in Figs. 5 and 6 for the membrane samples at 5 K. Fig. 7b shows that this dependence gradually levels out upon increasing the temperature until at and above ~200 K it completely vanishes. Needless to say that all the core complexes studied in this work behave likewise (data not shown). Similar behavior was previously observed for membranes of LH2 complexes [57,62,63], being explained by thermal averaging of various excitation pathways in the spectrally disordered membrane network.

4. Conclusions

In the present work, we systematically studied and interpreted the spectral and kinetic changes accompanying the assembly of the LH1 complex with the RC complex into core complexes of the photosynthetic purple bacterium *Rba. sphaeroides*. One of the prime motivations of this work was that the many literature data on the excitation energy transfer and trapping in photosynthetic bacterial membranes appear fragmented, irregular, and sometimes even controversial. This unsatisfactory situation is frequently vaguely assigned to "biological variability". The present work was set to make an order in at least some of the data by systematically studying a range of complexes from one and the same species, and using similar, carefully selected and controlled experimental conditions. As a result, the multiple factors that influence most the fluorescence lifetime of the core complexes, both in detergent-isolated and membrane-embedded form, were established, evaluated, and discussed, providing a more comprehensive understanding of the field. In the context of the current debate about the origin and the role of low-energy states in photosynthesis [51] we discovered a low-energy absorption tail present in the core complexes complete with the LH1 and RC complexes but absent in lone LH1 complexes. This tail, being accessible only at low temperatures, was identified as the special pair absorption band of the RC. We further showed that dimeric core complexes are more efficient quenchers of antenna exciton polarons than monomeric cores, confirming the recent discovery on full mutant chromatophore membranes of *Rba. sphaeroides*.

Transparency document

The Transparency document associated with this article can be found, in online version.

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