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Spectral properties of the photosynthetic reaction units reconstituted from bacterial reaction centres and antenna pigments located in liposomes suspended in buffer or ordered in Langmuir–Blodgett films

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Abstract

Artificial photosynthetic reaction units reconstituted from photosynthetic bacterial reaction centres and antenna pigments (bacteriochlorophyll *a* or chlorophyll *a*) and built in liposome vesicles were investigated. Samples were suspended in buffer or ordered in Langmuir–Blodgett films. The absorption, photoacoustic and delayed luminescence spectra of the liposomes in suspension and in Langmuir–Blodgett films deposited on quartz plates were measured. As a reference, suspensions or films containing reaction centres or antenna pigments only, both embedded in liposomes, were investigated. The absorption spectra of the samples containing reaction centres and pigment mixtures were the sum of the absorptions of samples containing reaction centres and pigments separately. This result shows that, in the mixed sample, strong interactions due to the formation of ground state aggregates are absent. The photoacoustic and absorption spectra are different because of the different yield of thermal deactivation of various chromophores. The yield of thermal deactivation of the pigments in reaction centres is higher than that of additional artificial antenna molecules. This yield is at least partially due to the rather efficient excitation energy transfer from the antenna to reaction centres competing with the thermal deactivation of excitation.

All samples exhibit a delayed luminescence with the spectra located in the spectral regions of the prompt fluorescence of the investigated pigments. A comparison of the intensity of delayed luminescence of the artificial photoreaction unit with the intensities of reference samples demonstrates that excitation energy transfer from the antenna to the chromophores of the reaction centres occurs.

In liposome suspensions and in monolayers, the absorption spectra of the samples containing reaction centres and pigment mixtures are similar, but the photoacoustic spectra and delayed luminescence spectra are different. This shows that the mutual interactions between the reaction centre chromophores and antenna pigments in liposomes located in suspensions and in monolayers are different. © 1997 Elsevier Science S.A.

Keywords: Bacteriochlorophyll; Chlorophyll; Delayed luminescence; Excitation energy transfer; Langmuir–Blodgett films; Lipids; Photoacoustic spectra; Reaction centres; Vesicles

1. Introduction

In photosynthetic organisms, the conversion process of light energy into charge separation is very efficient. Thus there have been several attempts to construct artificial systems to convert light energy into electrical energy using models which contain parts of photosynthetic organisms [1–3]. In such semi-natural arrangements, the use of reaction centres of photosynthetic bacteria, with artificially connected pigments, to work as light-harvesting systems has been proposed [4]. In a natural photosynthetic apparatus, the pigment–protein complexes are embedded in lipids which form biological membranes. Therefore, in the model used, both reaction centres (RCs) and antenna pigments were built together in liposomes.

In vivo, the photoresistance of pigments is realized by the attachment of chromophores to proteins [5]. In our proposed systems, the pigments and RCs are embedded into liposome

Abbreviations: Bchl, bacteriochlorophyll *a*; BPh, bacteriopheophytin; Chl, chlorophyll *a*; DHP, dihydromesochlorophyll; DPPC, L-α-Phosphatidylcholine dipalmitoyl; ET, excitation energy transfer; LB, Langmuir–Blodgett; LP, lipids; PAS, photoacoustic spectra; PRU, photosynthetic reaction unit; RC, reaction centre; *Rb. sphaeroides*, *Rhodobacter sphaeroides*

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vesicles. In such surroundings, the pigments and pigment–protein complexes are more stable than in solution [6].

It has been shown [4,7,8] that lipid vesicles are an excellent matrix in which it is possible to embed pigments and RCs without denaturation of the proteins and the formation of aggregates of the pigments.

From previous results [4] it follows that, in the reconstituted photosynthetic reaction unit (PRU), the excitation energy of the artificially reconstructed light-harvesting system is efficiently transferred to the chromophores of the RCs [4]. This result was obtained from fluorescence measurements. In addition, the charge transfer in such a system was shown by measurements of the light-induced absorbance changes and photocurrent generation [4].

The correct mutual orientation of the transition moments of the antenna pigments and chromophores located in RCs plays a crucial role in enabling efficient excitation energy transfer (ET) from the antenna to RCs. In natural systems, this correct orientation of the chromophores is ensured by the structure of the photosynthetic apparatus. We have attempted to find an orientation which enables efficient ET to occur by depositing liposomes containing RCs and pigments in monolayers. For this purpose, a second model system investigated in this work is constructed of eight or nine monolayers, containing the liposomes with the antenna pigments and RCs, deposited on a solid support. Monolayers of liposomes have also been investigated previously by Picard et al. [9] and Sui and Wang [10]. It has been established that, as a result of the addition of detergent, the vesicles of liposomes are broken and a flat monolayer is formed on the water subphase [10]. In our experiments, all samples containing RCs were solubilized by detergent and therefore probably contain broken vesicles forming a lipid layer (as shown in Fig. 1). In samples containing only pigments, closed but flattened liposome vesicles are preserved after Langmuir–Blodgett (LB) film formation. Chlorophyll *a* (Chl) or bacteriochlorophyll *a* (Bchl) were used as antenna pigments. LB films with liposomes containing only RCs or only pigment molecules were used as reference samples.

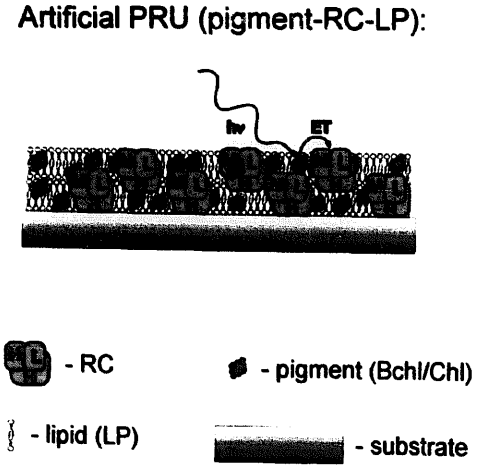

Fig. 1. Schematic illustration of LB system composed of pigments and RCs reconstituted into liposomes (Bchl/Chl–RC–LP).

Two artificial systems were constructed so that light energy absorbed by antenna pigments could be converted into electrical energy. This occurs because the charge in the RC is shifted and may be transferred to a proper electron acceptor. In the present stage of investigation of both models, we wish to establish only the conditions for efficient ET from the antenna to RCs. In this work, we investigate the pathways of pigment de-excitation competing with this process. Two important pathways of chromophore de-excitation involve the conversion of excitation into heat, i.e. thermal deactivation (TD) and trapping of the excitation followed by the emission of delayed luminescence. Therefore we have measured the photoacoustic spectra (PAS) and time-resolved delayed luminescence (DL) spectra. Fluorescence emission is also a competitive pathway of antenna pigment deactivation, but in the investigated LB film model this fluorescence signal was too low for measurement and the emission from the support plate was comparable with the fluorescence of the pigmented liposome layers. Therefore the fluorescence data concerning the previously investigated suspensions of the same liposomes [4] are used in the discussion of the DL spectra of the liposomes in monolayers.

A comparison of the spectral properties of the vesicles containing RCs and pigments located in suspension with those of the monolayers will reveal whether the interactions between RC chromophores and antenna pigments are changed by the deposition of liposomes in monolayers.

2. Materials and methods

2.1. Preparation of RCs and pigments

The RCs were isolated from the purple bacterium *Rhodobacter sphaeroides* (*Rb. sphaeroides*) carotenoidless mutant R-26 according to a modification of Clayton and Wang's method [11] described in detail in Ref. [4]. The RCs were solubilized using lauryl-dimethylamine-*N*-oxide (LDAO, 2%) in Tris-HCl buffer (10 mM, pH 8.2), and purified through sucrose solution layer and gel Sepharose CL-6B filtration. The final concentrations of the solubilized RCs ranged from 60 to 90 μM.

Chl was extracted from spinach and purified according to a procedure described by Katoh et al. [12]. Washed leaves were homogenized in acetone and precipitation of the pigments was obtained after solubilization in dioxan and chilled water. Carotenoids and pheophytins were removed by DEAE-Sepharose CL-6B and Chl *a* and Chl *b* were separated using Sepharose CL-6B column chromatography [4].

Bchl was extracted from *Rb. sphaeroides* R-26 chromatophores by solubilization in methanol, mixing with dioxan and precipitation by mixing with chilled water. Final purification of Bchl was performed using Sepharose CL-6B column chromatography [4,12].

2.2. Reconstitution of RCs and pigments into liposomes

The reconstitution of RCs and pigments into liposomes was carried out according to a modification of the procedure described by Mimms et al. [13]. Synthetic lipid L-α-phosphatidylcholine dipalmitoyl (DPPC, C16:0, Sigma Chemical Co., USA) was dissolved in chloroform with the addition of the appropriate concentration of pigment in an argon atmosphere and dried in vacuum to form a thin film deposited on the tube wall. The dried pigment–DPPC mixture was dissolved in buffer (10 mM Tris-HCl at pH 8.2) containing the appropriate concentration of RCs.

Sonication, freezing in liquid nitrogen, slow thawing to room temperature and repeated controlled sonication gave well-fused membranes [14]. As described previously in detail, this procedure leads to the production of small unilamellar vesicles in buffer solution with diameters in the range 200–350 Å. Finally, a concentration of RCs of 48 μM, with an RC/DPPC ratio of 1:800 and an RC/pigment ratio of 1:20, was obtained. In such a way, an artificial PRU reconstituted in lipid (LP) vesicles composed of DPPC, RCs and pigments, hereafter called pigment–RC–LP or, specifically, Bchl–RC–LP or Chl–RC–LP, was constructed. As a reference, liposomes containing only RCs (RC–LP) or only pigments (Bchl–LP or Chl–LP) with the same concentration were prepared.

2.3. Deposition of LB films

The deposition of LB films was carried out in an LB-5000 mini trough (KSV Instruments Co., Finland). Aqueous buffer solutions of pigment–RC–LP, RC–LP and pigment–LP were spread along a tilted glass rod on the water subphase Tris-HCl (2 mM, pH 8.2) containing $CaCl_2$ (2 mM) and sodium ascorbate (0.5 mM). After spreading, the material was incubated for 15 min until the detergent molecules had been solubilized into the subphase and the temperature was stabilized. Thereafter non-linear compression at 5 mm min^{-1} was carried out until a surface pressure of 25 mN m^{-1} was reached. The monolayer was transferred under controlled surface pressure to a hydrophilic quartz disc plate ($\Phi = 20$ mm). By means of the usual vertical deposition, only the first layer obtained showed a good transfer ratio; therefore the vertical deposition method was used only for the first deposition. For further depositions, the Y′-tape horizontal lifting method was applied [15]. For the latter method, a home-built offset angle substrate holder was added to the existing lifting instrument (KSV mini trough). The best results were obtained for a lifting speed of 2–3 mm min^{-1} (5 mm min^{-1} at vertical deposition) and a transfer pressure of 25 mN m^{-1}. Only the LB films deposited with a transfer ratio of about two in the case of horizontal lifting deposition and about unity for the first vertical deposition were used. All the measurements of the LB samples were performed immediately after deposition.

The addition of detergent to the sample containing RCs caused the breakage of the liposome vesicles which had been spread on the subphase (data not shown). Nine monolayers (one vertical and eight horizontal), formed from the destroyed liposome vesicles, were deposited on the support plate and, as a result, a layer was formed (schematically presented in Fig. 1). In the case of liposomes with only antenna pigments, the closed vesicles are still preserved on the subphase during film formation. For such samples, four double layers were deposited producing eight layers on the support plate. Therefore the structure of the reference sample containing only pigment molecules differs from samples with RCs, but in both types of sample pigment molecules are located in a lipid layer.

2.4. Spectral measurements

The absorption spectra were measured using a Specord M40 spectrophotometer (Carl-Zeiss Jena, Germany) with a computer on-line for data acquisition and handling.

Time-resolved DL spectra (in the microsecond range) were measured with an arrangement which has been described in detail previously [16]. The apparatus was equipped with a nitrogen and dye laser (type LN 120 C/LD2C, PRA Laser, Canada). The pulse duration was about 200 ps (full width at half-maximum (FWHM)). The intensity of the excitation light was sufficiently low to avoid nonlinear effects. A Hamamatsu photomultiplier R 928 was used as detector.

Under the experimental conditions used, it was possible to measure DL in the 600–900 nm spectral region. The samples containing Bchl were excited at 385 nm, while samples with Chl addition were excited at 425 nm. The DL spectra were measured in the time range from 0.2 μs to 100 μs after the laser pulse within a time window of 5 μs.

PAS were taken using a single-beam spectrometer built at the Poznań laboratory, based on the apparatus made at Trois-Rivières, Canada [17]. The xenon lamp used as light source enables spectra to be measured in the range 350–840 nm.

The PAS were measured with a light modulation frequency of 30 Hz and a phase shift between the modulated light beam and the measured photoacoustic signal of $\phi = 9.2°$. Carbon black was used as reference sample.

3. Results and discussion

3.1. Monolayers of liposomes

In order to check the process of monolayer formation, the dependence of the surface pressure on the LB film surface was investigated. The course of this dependence in all investigated cases is characteristic of well-formed LB films (Fig. 2), but the exact shapes of the curves are different for the different samples. It is known that liposomes can be destroyed by the presence of detergent [10]. Curves 1 and 3 in Fig. 2 differ because the detergent was added to samples with RCs during the purification procedure. These curves

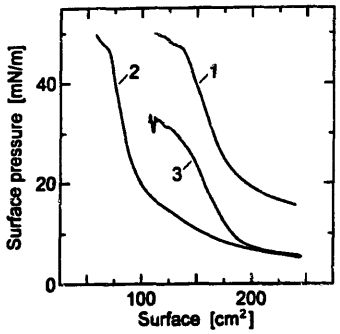

Fig. 2. π–A curves of LB monolayers: (1) Chl–RC–LP; (2) Chl–LP; (3) RC–LP (T = 18 °C).

show a sharp increase in the surface pressure, whereas the curve for the pigment alone (curve 2) exhibits a different pressure increase. The results observed can be explained by the different structures of the films in these two cases. The average area occupied by one lipid molecule for pigment–LP samples (without detergent), calculated from the known concentration of the spread chromophores, is more than four times higher than that of the (pigment–)RC–LP samples containing detergent. This means that the lack of detergent in pigment–LP counteracts the breakage of vesicles after spreading. Without the detergent (Fig. 2, curve 2) the vesicles remain intact; therefore monolayers as in Fig. 1 are not formed.

3.2. Absorption spectra

Fig. 3(a)–(c) illustrate the absorption spectra of the reconstituted PRU (pigment–RC–LP) in suspension and of the reference (RC–LP and pigment–LP) samples. The positions of the absorption, photoacoustic and DL bands, together with their assignments, for all measured samples are given in Table 1.

It follows from Fig. 3(c) and Table 1 that, in all spectra of the samples containing only RCs, characteristic maxima at 366 nm (B_Y transition of Bchl), 763 nm (Q_Y of bacteriopheophytin (BPh)), 808 nm (Q_Y of monomeric form of Bchl) and 870 nm (Q_Y of $Bchl_2$ dimer) are well resolved. The absorption of the RC and Bchl mixture (Bchl–RC–LP, Fig. 3(a)) is approximately the sum of the spectra of Bchl–LP (Fig. 3(b)) and RC–LP (Fig. 3(c)) reference samples (result not shown).

Fig. 4 shows the absorption spectra of the reconstituted PRU of LB layers (pigment–RC–LP; with the structure shown in Fig. 1) and the reference (RC–LP and pigment–LP) samples. Fig. 4(a)–(c) present the results for the samples containing Bchl and Fig. 4(d)–(f) for the samples containing Chl. The positions of the RC maxima (also given in Table 1) are similar for the suspension and LB films, and are in agreement with the literature data [5], which shows that the chromophores of the RCs do not interact strongly with the lipids.

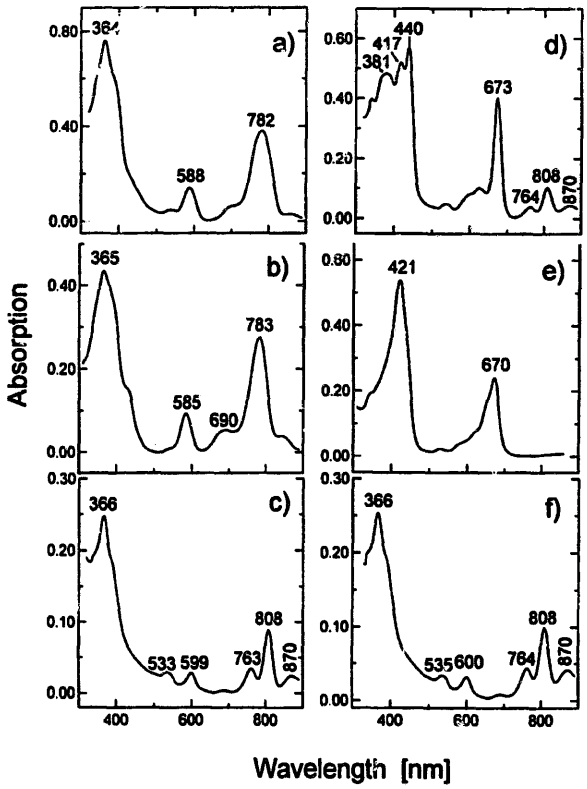

Fig. 3. Absorption spectra of liposome suspensions: (a) Bchl–RC–LP; (b) Bchl–LP; (c) RC–LP; (d) Chl–RC–LP; (e) Chl–LP; (f) RC–LP. n^{RC} = 4.8 μM, $n^{Bchl/Chl}$ = 96 μM, n^{LP} = 3.8 mM.

The absorption maxima of the LB films containing Bchl and Chl in lipids are similar in suspensions of vesicles and in monolayers (Table 1). The maxima are shifted about 10 nm towards longer wavelengths with respect to the positions of the maxima of the monomeric forms of these pigments in organic solvents [5,9]. This shift is due to the strong pigment–lipid interaction which preserves the formation of the pigment aggregates. The broad maximum at 788 nm observed in the Bchl–RC–LP film is formed by the overlap of the near-IR maxima of antenna Bchl in the lipid and in the RC. The weak absorption maximum at 690 nm probably belongs to the denaturated form of Bchl dihydromesochlorophyll (DHP) [18,19]. This maximum is low, which shows that Bchl is mostly in its native state. A similar situation was observed for systems pigmented by Chl (Fig. 4(d)–(f)). All RC maxima are well distinguished and maxima due to Chl absorption at 425 nm (B_Y) and 673 nm (Q_Y) are seen (Table 1). The position of the Bchl and Chl bands and their relative intensities are in agreement with the data reported for these pigments in LB films [9].

The spectra of both model systems containing a mixture of RCs and the given pigments are a superposition of the spectra measured for RCs and the pigments in separate samples. This result shows that strong interactions between RCs and pigment molecules due to ground state mixed aggregate formation do not occur. No new bands which could be attributed to such mixed aggregates are observed.

Table 1
Chromophore band positions in artificial PRU reconstructed in suspension and in LB films. Data for LB films in brackets

Type of sample	Measurement	Bchl					$Bchl_2$	BPh		Chl		DHP
		B_Y	B_X	Q_X	Q_Y	Q_Y^a	Q_Y	Q_X	Q_Y	B_Y	Q_Y	Q_Y
Bchl–RC–LP	ABS	364 [367]	[395s]	588 [585]	[788]	782[a] [788[a]]	[860]	[537]	[788]	–	–	[690]
	PAS			604 [598]	808	[795[a]]		550	772 [770]	–	–	692 [686]
	DL				[810]	[810[a]]			800	–	–	690 [690]
Bchl–LP	ABS	365[a] [367[a]]	[395[a]]	585[a] [585[a]]		783[a] [788[a]]	–	–	–	–	–	690
	PAS			596[a] [595[a]]		788[a] [795[a]]	–	–	–	–	–	692
	DL					800[a] [800[a]]	–	–	–	–	–	680
RC–LP	ABS	366 [367]	395s [395s]	599 [599]	808 [808]		870 [860]	533 [537]	763 [766]	–	–	
	PAS			608 [610]	808 [814]			550 [550]	776 [770]	–	–	
	DL				[820]				790 [790]	–	–	700 [700]
Chl–RC–LP	ABS	381 [367]	[395s]	[599]	808 [808]		870 [870]	535 [537]	764 [766]	417 [425]	673 [673]	
	PAS				808 [791]				768 [757]		682 [670]	
	DL								780 [790]		[690]	680 [690]
Chl–LP	ABS	–	–	–	–	–	–	–	–	421 [425]	670 [673]	
	PAS	–	–	–	–	–	–	–	–		678 [670]	
	DL	–	–	–	–	–	–	–	–		680 [690]	

[a] Maxima belonging to antenna Bchl pigments and bands representing the superposition of the contributions from the antenna and RCs.
ABS, absorbance maxima (nm); PAS, photoacoustic maxima (nm); DL, delayed luminescence maxima (nm) at an excitation wavelength of 385 nm (425 nm for Chl).

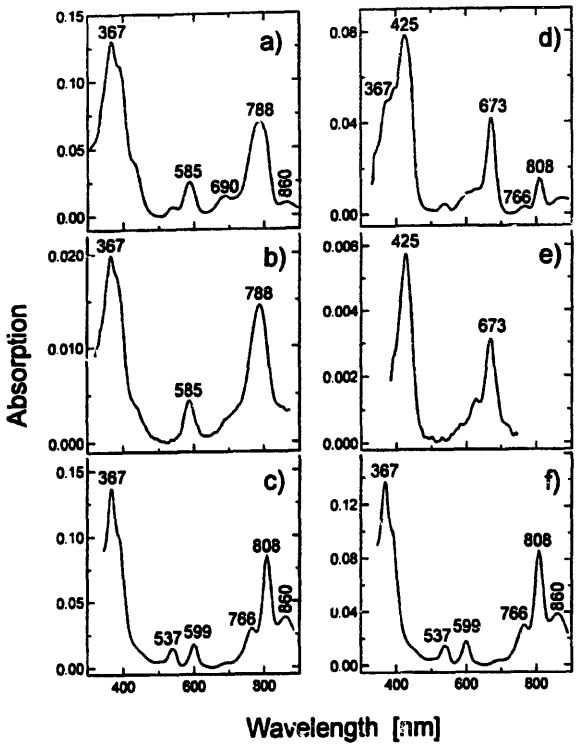

Fig. 4. Absorption spectra of nine (or eight) LB monolayers deposited on a quartz substrate: (a) Bchl–RC–LP; (b) Bchl–LP; (c) RC–LP; (d) Chl–RC–LP; (e) Chl–LP; (f) RC–LP.

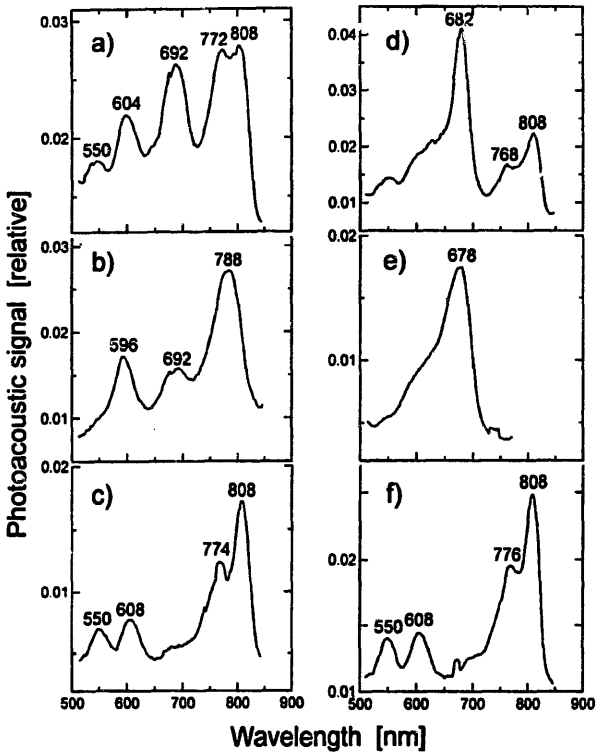

Fig. 5. PAS of liposome suspensions: (a) Bchl–RC–LP; (b) Bchl–LP; (c) RC–LP; (d) Chl–RC–LP; (e) Chl–LP; (f) RC–LP. $f = 30$ Hz, $\phi = 9.2°$.

3.3. Photoacoustic spectra

Fig. 5(a)–(c) show the PAS of a set of samples in lipid vesicle suspensions with the absorption shown in Fig. 3. In RC–LP (Fig. 5(c)), TD occurs with different efficiency in various chromophores. Deactivation is very efficient in the Bchl monomer (808 nm) and BPh (774 nm) absorption regions, but very inefficient in the absorption range of the special pair $Bchl_2$ (870 nm). As previously determined [20], the TD in the 875 nm region of whole bacteria was much lower than that in the Bchl monomer and BPh regions.

The PAS of Bchl–LP exhibits a Q_Y peak at 788 nm, i.e. shifted towards longer wavelengths with respect to the absorption maximum located at 783 nm (Fig. 5(b)). Such a shift is observed when various pools of molecules undergo different interactions with the surroundings and, as a result, exhibit various yields of TD. In whole bacteria immobilized in a stretched polymer film, the TD was different for different polarizations of the absorbed light, which shows that a different orientation with respect to the matrix chromophores leads to different TD [21]. Similar effects can occur in lipid membranes. In the PAS of the Bchl–RC–LP mixture (PRU), the peaks belonging to the RC chromophores, i.e. Bchl monomer (808 nm) and BPh (772 nm), are superimposed with the band of antenna Bchl (Fig. 5(a), Table 1). The high peak at 692 nm, which is seen as a low peak in absorption (Fig. 3(b)), may be due to DHP [18,19,21,22]. The ET from DHP to the RC chromophores seems to be inefficient and therefore this pigment gathers excitation energy and converts it into heat. This means that, to obtain an efficiently working model, we must avoid the denaturation of Bchl leading to the formation of DHP.

The PAS of the RC–LP reference sample (Fig. 5(f)) is similar to that shown in Fig. 5(c). The differences are within the limits of accuracy of sample preparation and PAS measurements. The PAS of Chl–LP (Fig. 5(e)) exhibits one main maximum at 678 nm shifted again with respect to absorption towards the long-wavelength region as observed in the Bchl sample (Fig. 3(a) and Fig. 5(a)). Mixing of RC with Chl (Chl–RC–LP) causes a higher PAS amplitude in the region of Chl absorption than the sum of both constituent PAS and also causes a further shift of the Chl maximum to 682 nm. This shows that Chl interacts with the RCs located in the lipid layer.

The PAS of LB films of artificial PRU (Fig. 6) differ strongly from the data for suspension samples (Fig. 5).

Fig. 6 shows the PAS of LB films of pigment–RC–LP and the reference samples. The comparison of the PAS of the artificial PRU system (Bchl–RC–LP) with the PAS of the reference samples (Bchl–LP and RC–LP) (Fig. 6(a)–(c)) shows that the maximum at 595–610 nm corresponds to Bchl Q_X in RCs, whereas that at 795 nm corresponds to the Q_Y transition of the antenna Bchl molecules interacting with lipids.

The main PAS maxima of the RCs, BPh (Q_Y) and monomer Bchl (Q_Y), are located at 770 nm and 814 nm respectively. In Bchl–RC–LP, the monolayer contribution to the PAS signal from antenna Bchl (795 nm) is much stronger than that from the Bchl monomer in RCs (814 nm). This

observation may suggest that TD in the artificial PRU occurs mostly in the free antenna pigments. However, the number of antenna molecules is about three times higher than that of monomeric Bchl in RCs. In addition, the strong overlap of the maxima of Bchl in liposomes with the maxima of Bchl located in RCs makes the interpretation equivocal. By comparing the amplitudes of PAS measured for Bchl–LP with the amplitudes of samples containing RC–LP, we can see that the chromophores of the RCs exhibit a much larger PAS amplitude than that of antenna Bchl in separate samples.

Such an interpretation is in agreement with the much clearer results concerning the samples pigmented by Chl (Fig. 6(d)–(f)). Chl gives a PAS maximum at about 670 nm. This maximum is also seen in the artificial PRU. The PAS signal is lower in the sample containing both pigment and RCs in LP than in the sample containing RC–LP alone. A similar situation was observed in absorption (Fig. 4(d)–(f)). The PAS maxima due to RC–LP are located as described previously (Fig. 6(c), Fig. 6(f) and Table 1), but the shape of the PAS maximum of RC in the presence of Chl is deformed, perhaps because the efficiencies of ET from the antenna to the various chromophores of RCs are different. The change in the amplitude and shape of PAS for the sample with Chl and RC, together with that of RC alone, may be due to the non-uniform feeding of the various chromophores of the RCs by the energy absorbed by Chl. The various chromophores of RCs have different yields of TD [22]. Comparing the PAS results for the monolayers (Fig. 6) with those for the liposome suspension (Fig. 5), we can see strong differences in the antenna–RC interactions. In suspension, the shape of the PRU PAS differs strongly from the sum of the PAS of the separate RC and pigment samples; this is not so for the PAS of similar samples in LB films.

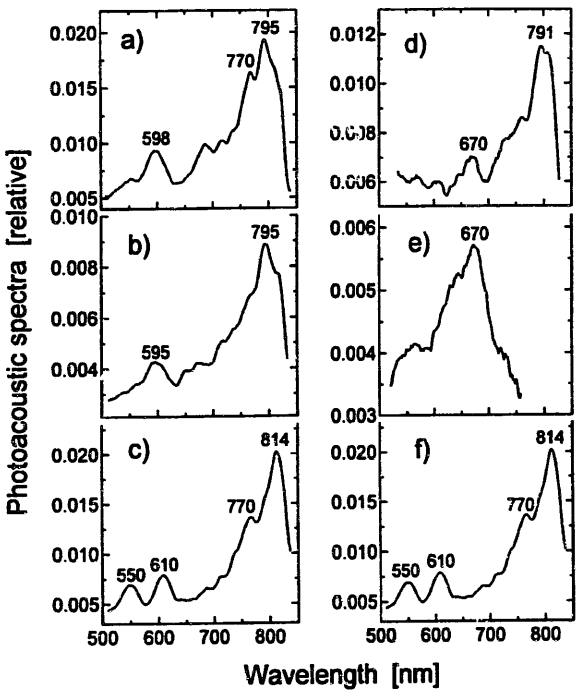

Fig. 6. PAS of nine (or eight) LB monolayers deposited on a quartz substrate: (a) Bchl–RC–LP; (b) Bchl–LP; (c) RC–LP; (d) Chl–RC–LP; (e) Chl–LP; (f) RC–LP. $f = 30$ Hz, $\phi = 9.2°$.

The yield of TD of the antenna pigments is much lower for both models than the yield of TD of RC chromophores. If we take into account the ratio of the number of antenna pigments to the number of RCs (20:1), which creates a ratio of chromophore molecules of 20:6 (20 pigments surrounding RC for the six pigments in RCs), TD from Bchl in RC is more effective than that in the antenna. This means that the energy delivered to RCs (which is not transformed into electron transfer) is transformed mostly into heat, whereas pigments embedded directly in the lipid layer deactivate energy by other pathways, at least partially by ET to RC. This observation shows that the pigment–protein structure deactivates the excitation energy into heat more efficiently than the pigment–lipid bilayer.

3.4. Delayed luminescence spectra

Fluorescence spectra for liposome suspensions have been reported previously [4]. It was not possible to measure the fluorescence spectra of LB monolayer samples, because of the small amount of pigment molecules in the eight or nine monolayers and because of the superposition of the support plate luminescence [23]. The DL spectra are more easily observed, because the opportunity exists to cut out the scattered excitation light and support plate emission by an appropriate choice of time window. The DL provides information about that part of the excitation light which is trapped by the investigated systems. For model systems containing Chl molecules, DL in the spectral region similar to that of fluorescence is usually observed [19,22,23]. This observation is due to the so-called β-DL (thermal activation from triplet to excited singlet state) and/or by the recombination of ionized pigment molecules leading to the excited singlet state [24].

Fig. 7 shows the DL spectra of the artificial PRU of lipid vesicle suspensions. In the spectrum of RC–LP (Fig. 7(c)), two well-resolved maxima are observed: that located at 790 nm is clearly due to BPh emission; that at 700 nm could be the emission of DHP. For the RC of the same mutant of *Rb. sphaeroides*, a similar DL spectrum was observed with maxima at 790 nm and 690 nm [19]. The last maximum was described as belonging to DHP. The observed 10 nm shift between these data may be due to chromophore interactions with lipids. The PAS maximum of this form was, in our experiments, about 692 nm (Table 1); therefore the Stokes shift is rather low. For an RC of *Rb. sphaeroides* containing carotenoids, the DL exhibits only one maximum due to BPh. This shows that carotenoids prevent the production of DHP.

Bchl–LP (Fig. 7(b)) exhibits, in the DL spectrum, one maximum at 800 nm due to the emission of Bchl molecules, which is related to the 783 nm absorption. The DL intensity of this pigment is much higher than that of RC–LP. In the RC–Bchl–LP mixture excited at 385 nm in the region of Bchl absorption, the DL spectrum (Fig. 7(a)) is similar to that of Bchl alone, but the peak is much wider than in Fig. 7(b).

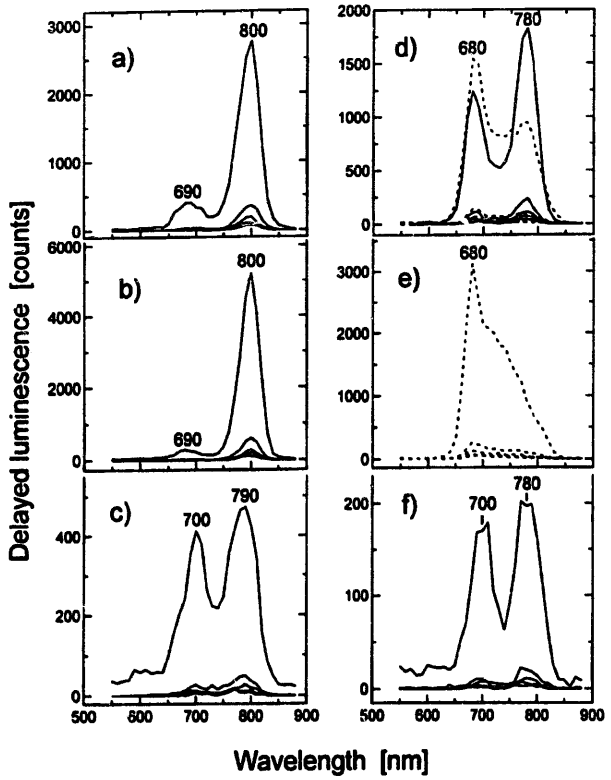

Fig. 7. DL spectra of liposome suspensions: (a) Bchl–RC–LP; (b) Bchl–LP; (c) RC–LP; (d) Chl–RC–LP; (e) Chl–LP; (f) RC–LP. $\lambda_{exc}=385$ nm (broken curves, $\lambda_{exc}=425$ nm). Curves represent time windows: (1) 0.2–5.0 μs; (2) 5–10 μs; (3) 10–15 μs; (4) 15–20 μs; (5) 20–25 μs. Concentrations of RC and chromophores as in Fig. 3.

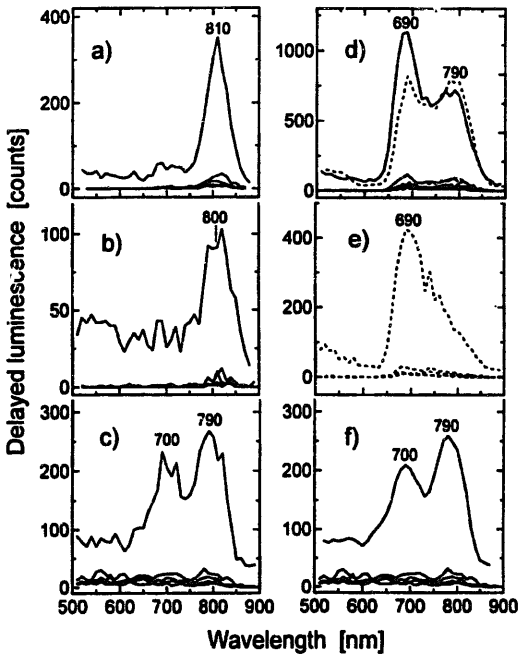

Fig. 8. DL spectra of nine (or eight) LB monolayers deposited on a quartz substrate: (a) Bchl–RC–LP; (b) Bchl–LP; (c) RC–LP; (d) Chl–RC–LP; (e) Chl–LP; (f) RC–LP. $\lambda_{exc}=385$ nm (broken curves, $\lambda_{exc}=425$ nm). Time windows as in Fig. 7.

This peak is probably due to the superposition of BPh from RC and antenna Bchl. The second peak at 690 nm is due to DHP. The DL intensity of this sample is lower than that of Bchl–LP. This shows the effective ET from the strongly emitting Bchl to the weakly luminescent RC.

The set of samples pigmented by Chl is easier to interpret than the Bchl antenna system, because of the smaller overlap of the RC chromophores and the added pigment spectra. It follows from Fig. 3(d)–(f) that, in the absorption spectrum of the Chl–RC–LP mixture, the absorption of Chl (at 673 nm) and the three maxima characteristic of RC at 764 nm (BPh), 808 nm (Bchl monomer) and 870 nm (special Bchl pair) are well resolved. However, it seems that there is some interaction of Chl with RC because the position of the Chl absorption is shifted from 670 nm, which was observed in the Chl–LP sample, to 673 nm in the mixture. In addition, the half-bandwidths decrease. All the DL spectra of RCs in the lipid are similar (Fig. 7(c) and Fig. 7(f)). Chl–LP alone, on excitation at 425 nm, exhibits DL with a maximum at 680 nm, with a strong shoulder on the long-wavelength side of the main band (Fig. 7(e)). At the same excitation wavelength, predominantly exciting Chl molecules, the sample with RC and Chl in lipid vesicles (Fig. 7(d), broken line) exhibits a higher maximum at 680 nm and an additional maximum at 780 nm. This spectrum is the superposition of Chl and RC emission. RCs are excited predominantly by ET from Chl, because the emission of RC chromophores is stronger than in the sample with RC alone. At an excitation wavelength of 385 nm, both RC chromophores and Chl are excited. In this case, the maximum at 780 nm, attributed to BPh located in RC, is higher than that at 680 nm, due to Chl and DHP emission. Due to the superposition of the absorption bands in the Soret region, it is not possible to establish quantitatively the yields of ET between the added antenna pigment and the various chromophores in RC. However, the increase in the intensity of emission due to RC chromophores as a result of Chl addition shows clearly that some excitation energy is transferred from the artificially added Chl antenna to RCs.

Fig. 8 presents the DL spectra of LB films of Bchl–RC–LP (Fig. 8(a)) and of the reference samples (Bchl–LP and RC–LP, Fig. 8(b) and Fig. 8(c) respectively). The DL of RC–LP shows, as in Fig. 7(c) and Fig. 7(f), maxima at about 700 nm and 790 nm.

Bchls alone embedded into lipids in an LB film give a rather weak DL maximum at about 800 nm (Fig. 8(b)). This result differs from that observed for vesicle suspensions. This difference may be due to the different arrangement of pigment transition moments. The DL spectrum of PRU shows a single strong maximum at 810 nm, which can be attributed to BPh in RC and/or to Bchl antenna in liposomes. After comparing the amplitudes of the DL signals (Fig. 8(b) and Fig. 8(c)), the first supposition seems to be more plausible. At 700 nm, the DHP in RC exhibits a band which practically vanishes in PRU (Fig. 8(a)). The narrower DL band of PRU (Fig. 8(a)) compared with the RC band (Fig. 8(c)) sug-

gests ET from the antenna to a special type of chromophore in RC.

The fluorescence of the same type of suspended PRU was previously observed [4], with the antenna Bchl maximum at 790 nm. The 10 nm shift of the maximum between prompt fluorescence and DL suggests that different chromophores in RCs take part to various extents in the fluorescence and DL emission.

Fig. 8(d)–(f) show the DL spectra for LB films pigmented by Chl. Two main maxima are observed in the PRU spectrum: the first at 690 nm, belonging (Fig. 8(e)) to Chl antenna pigments, and the second at 790 nm, due probably to BPh in RC (Fig. 8(f)). From the previously measured prompt fluorescence, the action spectra of the same Chl–RC–LP suspension [4] at both excitation wavelengths show that the system is excited with different efficiencies. The much stronger emission in the 790 nm region for the PRU than for the RC–LP sample, observed on 425 nm excitation, shows that ET occurs from the antenna to RC chromophores.

The decay time of the observed DL ranges from 22 μs to 27 μs according to monoexponential decay.

4. Conclusions

1. Antenna pigments interact with the lipids of liposomes and therefore do not form aggregates. The interactions of RC chromophores with lipids are much weaker, but their interactions with proteins also prevent aggregation.
2. Ground state mixed aggregation of RC and pigments is not observed, but mutual interactions follow from the PAS and DL results. These interactions are different in liposome suspensions and in monolayers.
3. The TD of chromophores in RCs is more efficient in most cases than TD in added antenna pigments.
4. TD of Chl in PRU is different from that of the pigment alone in liposomes (Chl–LP). This confirms the interaction of excited Chl from the antenna with RCs.
5. The shape of PAS measured for PRU in suspensions is different from the sum of the PAS of RC and pigments in separate samples. This shows that ET to various chromophores can be different. This effect is less visible in monolayers.
6. All samples exhibit DL in the spectral regions of the prompt fluorescence of their pigments. This may be β-DL (thermal activation from triplet to excited singlet) and/or the result of pigment ionization and delayed recombination. The decay time of DL was in the range 20–30 μs.
7. From the intensities of DL of PRU compared with the intensities of DL of the reference samples, it can be concluded that ET from the antenna to the chromophores of RCs occurs.
8. PRU in suspensions and in monolayers differ in their spectral properties due to the different mutual orientation of the chromophores in these cases.

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