ELSEVIER

Journal of Photochemistry and Photobiology A: Chemistry 116 (1998) 147–151

Journal of Photochemistry and Photobiology A:Chemistry

The interactions between bacteriochlorophyll *c* and amphiphilic peptides

Jun Miyake [a], Toshinori Kusumi [a], Alina Dudkowiak [a], Jacek Goc [b] Danuta Frąckowiak [b,*]

[a] *National Institute for Advanced Interdisciplinary Research, AIST/MITI, 1-1-4 Higashi, Tsukuba, Ibaraki 305, Japan*

[b] *Molecular Physics Division, Institute of Physics, Poznań University of Technology, ul. Piotrowo 3, 60-965 Poznań, Poland*

Received 12 January 1998; accepted 11 May 1998

Abstract

Absorption, fluorescence, photoacoustic and delayed luminescence spectra of bacteriochlorophyll *c* with and without peptides located in dimethyl sulfoxide were measured. For most molecules, the hydrophobic interactions between peptide and pigment in the ground state seems not to be very strong, as it follows from the absorption spectra of the samples with and without peptides. From photoacoustic spectra, it seems that at least part of the illuminated pigment molecules are forming complexes with peptides. These complexes are very efficient in thermal deactivation of excitation. The occurrence of the interactions between peptides and bacteriochlorophyll *c* follows also from delayed luminescence spectra, which are different for the sample with and without peptides. The observed delayed luminescence is so-called delayed fluorescence or E-type delayed luminescence because it is generated by thermal activation from the triplet to first excited state followed by emission from this state.

Keywords: Bacteriochlorophyll *c*; Peptides; Delayed luminescence; Delayed luminescence decay; Photoacoustic spectra; Fluorescence; Low temperature spectra

1. Introduction

It is known that for the creation of giant antenna complexes (chlorosomes) in green sulfur bacterium *Prostecochloris aestuarii* is responsible predominantly the interaction between bacteriochlorophyll *c* (Bchl) molecules [1,2]. Bchl is a main pigment of these complexes. Some interactions between pigment molecules and proteins cannot be excluded [3]. In other organisms the most of chlorophyll molecules are bound to proteins. The pigments are surrounded by hydrophobic amino acids in such proteins. In order to investigate of the hydrophobic pigment–peptide interactions in green bacteria, the spectral properties of Bchl solutions in the presence and absence of peptides were examined. A peptide with α-helical structure with the part of the hydrophobic molecular surface was synthesized. The absorption, fluorescence, time resolved delayed luminescence at various temperatures as well as the photoacoustic (PA) spectra were measured.

2. Material and methods

Bchl was obtained from *P. aestuarii*. Extraction, purification and identification procedures were described previously [4].

Peptide, Pep-1 (Ac-(KLLEELK)$_4$-(ELK)-NH$_2$) is an α-helical amphiphilic 32-residues peptide. It is designed to have hydrophobic and hydrophilic surfaces along the main chain when these peptides are formed an α-helix (Fig. 1). Pep-1 was synthesized by peptide synthesizer (9050 Plus Pep-Synthsizer, Perceptive) using Fmoc (9-Fluorenyl-methoxy-carbonyl) solid phase method [5] on PEG-PS resin. Cleavage from the resin and deprotection of side chains were achieved with a trifluoroacetic acid (TFA) method. Crude peptides were purified by a reverse-phase high performance liquid chromatography (Shimadzu Japan) with octadecyl column (Waters USA) with gradient elution method using acetonitrile and water both containing 0.1% TFA [6]. The eluent was freeze-dried. The molar absorption coefficient of Pep-1 ($\varepsilon = 1.64 \times 10^4$ l mol^{-1} cm^{-1}) was evaluated by dry weight method. Pep-1 has a high content of α-helix (more than 75%) as it was checked by CD measurements (unpublished data).

The Bchl ($c = 4 \times 10^{-4}$ M) with Pep-1 or without peptides were dissolved in dimethyl sulfoxide (DMSO). Absorption spectra were measured using a Specord M40 spectrometer (Zeiss, Germany). Steady-state fluorescence spectra were measured by homemade arrangement. As a receiver, a photomultiplier R928 (Hamamatsu Japan) was used. Delayed luminescence (DL) was measured with an apparatus

* Corresponding author. Tel.: +48-61-8782344; fax: +48-61-8782324; e-mail: frackow@phys.put.poznan.pl

1010-6030/98/$ - see front matter
PII S1010-6030(98)00296-2

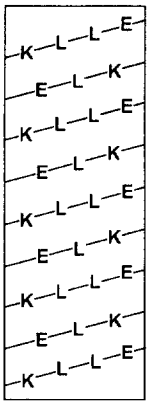

Fig. 1. Sequence of Pep-1.

described elsewhere [7]. The excitation light source was a combination and a nitrogen and dye laser (Type LN1120C/LD2C, PRALaser). Pulse duration was 22 ps (full width at half maximum, FWHM). Signals were gathered in a multichannel analyzer and computer processed. All DL spectra were taken with an additional delay of 200 ns with respect to the laser peak in order to eliminate the prompt fluorescence contributions. As a receiver, photomultiplier R2658P (Hamamatsu Japan) was used. All emission spectra were corrected on the spectral sensitivity of receiver and the monochrometer output. In order to measure low temperature DL spectra, the sample was located in cryostat (closed cycle refrigeration systems model TSL-22, Cryophysics Switzerland). The PA spectra were measured with a single-beam spectrometer constructed at Poznan University of Technology [8].

3. Results and discussion

Fig. 2 shows the absorption spectra of Bchl with and without Pep-1 dissolved in DMSO. Both spectra are very similar, characteristic for monomer Bchl. The maxima of Bchl in DMSO are seen at 664 nm and 437 nm, it means they are slightly shifted with respect to the Soret and Qy band located at 668 nm and 435 nm, respectively, observed in chloroform [9]. From the absorption spectra, the interactions between Bchl and Pep-1 are almost not seen. The Soret band is shifted only from 435 nm to 437 nm and red band is still at 664 nm. Also, the aggregation of pigment is not observed. The aggregates of Bchl exhibit usually absorption in long wavelength region; e.g., for tetrameric form at about 721 nm [10].

Fig. 3 shows the fluorescence spectra of the same set of samples upon excitation at 435 nm (Fig. 3a) and at 452 nm (Fig. 3b). In both samples, Bchl exhibits the main maximum at 672 nm with a shoulder at 731 nm (Fig. 3a). The fluorescence spectrum of Bchl with Pep-1 in DMSO exhibits, at 435 nm excitation, very similar shape to that one observed for Bchl alone but the ratio of the main maximum to the shoulder increases. This effect is stronger at 452 nm excitation (Fig. 3b), characteristic for pigment aggregates absorption region.

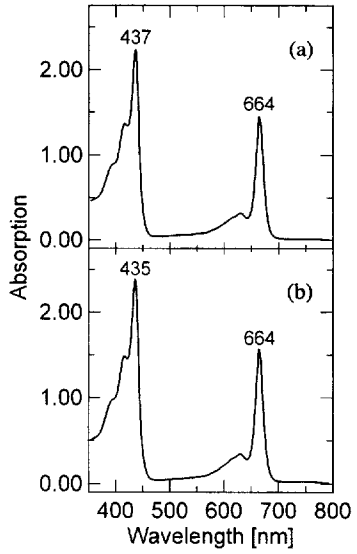

Fig. 2. Absorption spectra of: (a) Bchl in DMSO; (b) Bchl+Pep-1 in DMSO.

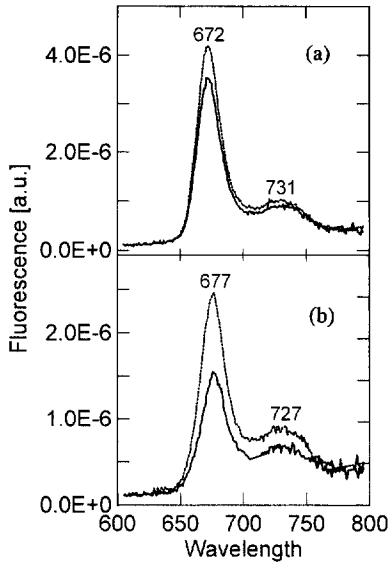

Fig. 3. Fluorescence spectra of: Bchl+Pep-1 in DMSO (solid line) and Bchl in DMSO (dashed line). Excitation wavelength: (a) $\lambda_{exc}=435$ nm; (b) $\lambda_{exc}=452$ nm.

Both samples at such excitation, have the main maximum shifted to 677 nm with the shoulder at 727 nm. The intensity ratio of shoulder to main maximum is higher for Bchl alone than for a pigment with peptide. The long wavelength emission at 727–731 nm region could be due to tetrameter emission [10], but it seems that the presence of peptides preserves the tetramer formation.

Fig. 4 presents the PA spectra of Bchl without and with peptides dissolved in DMSO. To exhibit maxima corresponding to the pigment absorption in a case of a sample without polypeptides, it was necessary to apply high (4×10^{-3} M) pigment concentration. The obtained PA spectrum shows strong signal around 580 nm region (Fig. 4a). This PA signal has to be due to some strongly thermally deactivating mole-

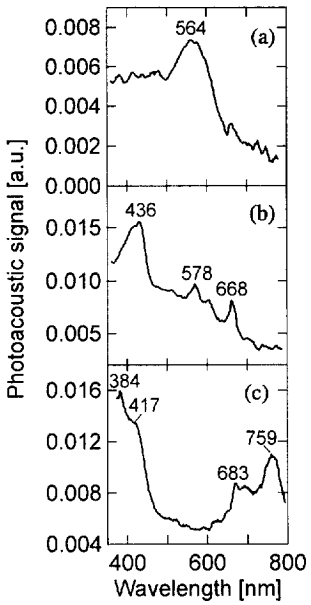

Fig. 4. Photoacoustic spectra of: (a) Bchl in DMSO, Bchl concentration $c=4\times10^{-4}$ M, modulation frequency $f=12$ Hz; (b) Bchl in DMSO, $c=4\times10^{-3}$ M, $f=16$ Hz; (c) Bchl+Pep-1 in DMSO, $c=4\times10^{-4}$ M, $f=12$ Hz. Phase shift $\Delta\varphi=160°$.

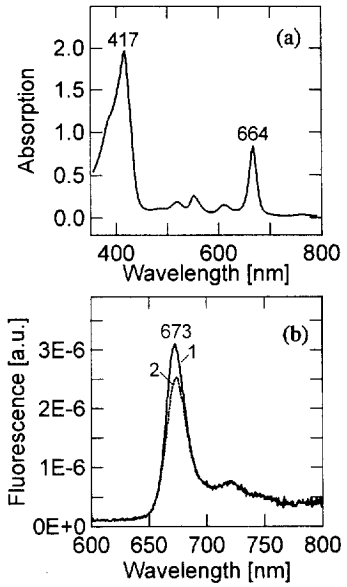

Fig. 5. Absorption (a) and fluorescence (b) spectra of Bchl+Pep-1 in DMSO after PA spectra measurements. Wavelength of excitation: (1) $\lambda_{exc}=417$ nm, (2) $\lambda_{exc}=435$ nm.

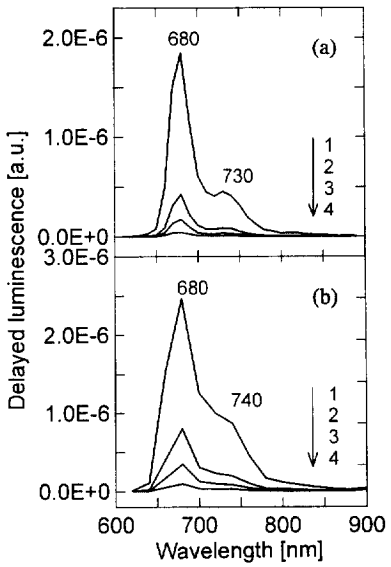

Fig. 6. Delayed luminescence spectra of: (a) Bchl in DMSO; (b) Bchl+Pep-1 in DMSO. Excitation wavelength $\lambda_{exc}=435$ nm, temperature $T=293$ K. Spectra taken at time windows: (1) 0.2–5.2 μs; (2) 5.2–10.2 μs; (3) 10.2–15.2 μs; (4) 15.2–20.2 μs.

cules or molecular complexes practically not observed in absorption (Fig. 2a). It was found that some porphyrins are forming aggregates with the DMSO participation [11]. In dry DMSO, this solvent molecules can intercalate between two pigment molecules forming aggregates. In DMSO–water binary solvent, it is a much more complex situation, because the ionic forms of dye are created. Of course, it is not a close analogy between metal free porphyrins and Bchl, but the participation of some pigment molecules in such interaction cannot be excluded. The unexpected shape of the PA spectrum of Bchl *c* in DMSO can be due to slowly thermally decaying excited pigment–DMSO complexes. It is decreasing with the increase in modulation frequency. In a case of the sample with polypeptides PA signal is much more similar to the absorption spectrum (Fig. 2b) than in a case of solution without Pep-1 (compare Fig. 2a and Fig. 4a). In a red region are seen two maxima: at 683 nm and at 759 nm. Last one has to be done by large aggregates [10]. It is not excluded that light used for PA measurements can partially pheophytinized the sample. As a result of the prolongated illumination (Fig. 5) in the Soret region, the maximum at 417 nm is observed. But the differences in PA spectra, between samples without (Fig. 4a) and with peptide (Fig. 4b) are not caused by sample degradation. All samples were irradiated in the same time by the same light intensities.

Fig. 6 shows the DL spectra excited at 435 nm. The main maxima of DL spectra for Bchl in both samples without and with Pep-1 are located at 680 nm, it means very close to the prompt fluorescence region. The spectrum shapes of samples with and without peptides are different. The ratio of the shoulder (730 nm or 740 nm) to the main band is higher for the sample with peptides. It is an opposite situation to that observed for prompt fluorescence (Fig. 3). The intensity of the main maximum of the sample with peptides is higher than for Bchl alone. It suggests that Bchl–DMSO complexes are not emitting DL. In both cases, the DL is decaying rather slow: but for Bchl alone decay is slower (8.0 μs) than for mixture with peptides (5.5 μs, Table 1). The semilogarithmic plot of ln intensity vs. the time after the excitation does not exactly show linear dependence (Fig. 7), suggesting that the contributions from the molecules located in slightly different surroundings occur.

Fig. 8 presents the dependence of DL intensity (in ln scale) as a function of temperature. The DL signal always decreases

Table 1
Delayed luminescence decay times for Bchl in DMSO and Bchl + Pep-1 in DMSO at $\lambda_{em} = 680$ nm, $\lambda_{exc} = 435$ nm

Sample	Bchl in DMSO		Bchl + Pep-1 in DMSO	
T (K)	τ (s)	RMS	τ (s)	RMS
203	9.4	0.985	4.9	0.982
253	7.2	0.988	3.9	0.920
293	5.5	0.964	5.6	0.993

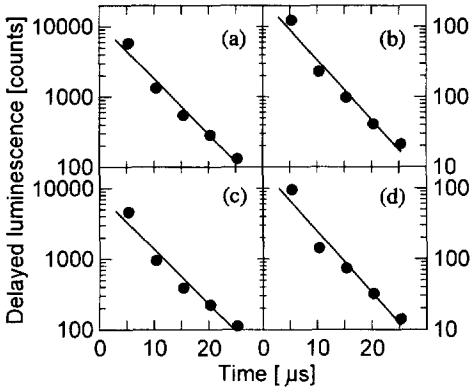

Fig. 7. Delayed luminescence decay in semilogarithmic scale ($T = 293$ K, $\lambda_{exc} = 435$ nm) of: (a) Bchl in DMSO, emission wavelength $\lambda_{em} = 680$ nm, lifetime $\tau = 5.5$ μs; (b) Bchl in DMSO, emission wavelength $\lambda_{em} = 730$ nm, lifetime $\tau = 5.1$ μs; (c) Bchl + Pep-1 in DMSO, emission wavelength $\lambda_{em} = 680$ nm, lifetime $\tau = 5.6$ μs; (d) Bchl + Pep-1 in DMSO, emission wavelength $\lambda_{em} = 730$ nm, lifetime $\tau = 5.0$ μs.

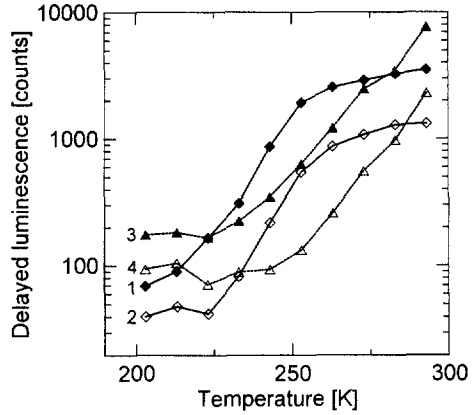

Fig. 8. Temperature dependence of delayed luminescence emission (natural logarithmic scale, $\lambda_{exc} = 435$ nm): Bchl in DMSO, emission wavelength (1) $\lambda_{em} = 680$ nm, (2) $\lambda_{em} = 730$ nm; Bchl + Pep-1 in DMSO, emission wavelength (3) $\lambda_{em} = 680$ nm, (4) $\lambda_{em} = 730$ nm.

with the sample cooling. This decrease has a slope similar for a main maximum (at 680 nm) and for the shoulder (at 730 nm), but this slope is different for pigment with, than without Pep-1. The slope is steeper for the pigment alone than for a pigment with Pep-1, and the Bchl alone exhibits different changes in a region of higher temperature (250–300 K) than the sample with peptides.

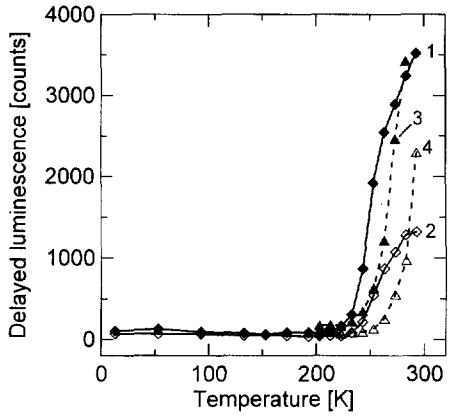

Fig. 9. Temperature dependency of delayed luminescence emission: The description of curves is as in Fig. 7.

The decrease in the DL intensity with the sample cooling shows that the thermal activation is necessary for the generation of this emission. It is even more clear from Fig. 9, where the DL intensity vs. temperature, for the wider temperature range, is plotted. The DL emission is practically not measurable for the temperature below 200 K. Such situation is observed till the lowest measured temperature (13 K). Such temperature dependence, as shown in Fig. 9, is typical for the DL emission thermally activated from the triplet to the first excited state followed by emission from this excited singlet. This kind of DL is so-called delayed fluorescence or according to Birk's [12] E-type delayed luminescence.

We cannot observe phosphorescence, i.e., the radiative emission from the triplet to the ground singlet state. Such emission was observed by Krasnovsky [13] for Bchl in polymethacrylate and in the frozen solutions of this pigment. The maximum of phosphorescence was located at 960 nm [13]. The positions of the absorption, fluorescence and DL bands are not dramatically dependent on the type of solvent [4,9,10,13]; therefore, the triplet state of Bchl in DMSO is probably located in a similar energy region as it was observed for this pigment in other media [13]. At such supposition, the energy difference between the lowest triplet and the first excited singlet should be about 0.55 eV. This energy gap is similar to that observed for other dyes [12]. As it follows from Figs. 8 and 9, such energy gap (about 9×10^{-20} J) can be thermally activated using the temperatures higher than 200 K. The $k_B T$ value calculated for 200 K is 2.8×10^{-21} J, whereas for 293 K it is equal to 4.0×10^{-21} J. It is clear that the value of $\Delta k_B T$ is comparable with the energy gap which is about 9×10^{-20} J. This energy gap cannot be crossed for lower temperature than 200 K (Figs. 8 and 9). The difficulty in the observation of phosphorescence of our samples can be due to the quenching of this emission by the oxygen, or by the interaction with solvent which is strongly interacting with the pigment, as it follows from the PA spectra (Fig. 4a). The same quenching of course concern the DL, but because it is more intensive, it could be observed using our set-up. As it follows from Krasnovsky results, the phosphorescence rate is several order lower than that of DL [13]. The lifetime of

phosphorescence observed by Krasnovsky was about 1 ms whereas the lifetime of DL of our sample was from 5 to 9 μs.

4. Conclusions

The hydrophobic interactions between pigment and peptide was confirmed by PA spectra measurements. In a sample without polypeptide at least part of illuminated pigment molecules are forming with DMSO complexes, which are very efficiently thermally deactivating excitation energy, whereas such effects are not observed in a sample with polypeptides.

The different spectra and decay times of DL of samples with and without polypeptides support such conclusion.

In a ground state, the interactions between peptides and pigment seems not to be very strong, as it follows from similar absorption spectra of Bchl with and without peptides.

Observed DL is generated by thermal activation from the triplet to the first excited state followed by emission from this state in time range from 5 μs to 9 μs; therefore, it is called delayed fluorescence or E-type DL.

Abbreviations

Bchl	Bacteriochlorophyllc
DL	Delayed luminescence
DMSO	Dimethyl sulfoxide
FWHM	Full width at half maximum
LH	Light harvesting
PA	Photoacoustic
Pep-1	α-Helical amphiphilic peptide
TM	Transition moment
K_B	Boltzmann constanty
RMS	Residual mean square

Acknowledgements

The paper was done in a frame of Japan–Poland Cooperation Joint Project No. 3 (RJ-3). J. Miyake, T. Kusumi and A. Dudkowiak were supported in part, under 'The Bionic Design Project' of National Institute for Advanced Interdisciplinary Research, AIST/MITI. D. Frąckowiak and J. Goc were supported by State Committee for Scientific Research (Polish KBN grant 1997/98 6PO4A019).

References

[1] A.J. Hoff, J. Amesz, in: H. Scheer (Ed.), Chlorophylls, CRC Press, Boca Raton, FL, 1991, p. 723.
[2] A.R. Holzwart, K Griebenow, k Schaffner, Z. Naturforsch. 45 (1990) 203.
[3] K. Matsuura, M. Hirota, K. Shimada, M. Mimuro, Photochem. Photobiol. 57 (1993) 92.
[4] A. Dudkowiak, C. Francke, J. Amesz, Photosynth. Res. 46 (1996) 427.
[5] L.A. Caprino, Y. Han, J. Org. Chem. 37 (1972) 3404.
[6] H.P. Bennett, C.A. Browne, S. Solomon, Biochemistry 20 (1981) 4530.
[7] A. Planner, D. Frąckowiak, Photochem. Photobiol. 54 (1991) 445.
[8] D. Ducharme, A. Tessier, R.M. Leblanc, Rev. Sci. Instrum. 50 (1979) 42.
[9] A. Dudkowiak, C. Francke, J. Amesz, A. Planner, I. Hanyż, D. Frąckowiak, Spectrochim. Acta Part A 52 (1996) 251.
[10] A. Dudkowiak, C. Francke, J. Amesz, A. Planner, D. Frąckowiak, Spectrochim. Acta Part A 52 (1996) 1661.
[11] R.M. Ion, International Symposium on Photodynamic Therapy, Wroclaw, 1997.
[12] J.B. Birks, Photophysics of Aromatic Molecules, Wiley-Interscience, London, 1970, 372.
[13] A.A. Krasnovsky Jr., P. Cheng, R.E. Blankenship, T.A. Moore, D. Gust, Photochem. Photobiol. 57 (1993) 324.