The influence of rigid matrices on the processes of deactivation of excited bacteriochlorophyll c

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Abstract

Bacteriochlorophyll c is a main pigment of green photosynthetic bacteria which is occurring in these organisms in various states of aggregation. Spectral properties such as absorption, fluorescence, delayed luminescence and photoacoustic spectra of bacteriochlorophyll c in two rigid matrices: silica glass and polymethylmethacrylate were established. The different photoproducts and aggregates of bacteriochlorophyll c exhibit various yields of thermal deactivation. Absorptions of some photoproducts are similar therefore the photoacoustic spectra are very useful in the investigations of the photochemical reactions. The pigments are more photochemically stable in silica glass than in polymer, which is due to the efficient quenching of triplet states in the silica glass.   © 1998 Elsevier Science S.A. All rights reserved.

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1. Introduction

Bacteriochlorophyll c (BChl c) occurs in giant antenna complexes —chlorosomes of photosynthetic green bacteria in a highly aggregated state [1–3]. The structure of chlorosome rods is due predominantly to pigment–pigment interactions but some role of proteins is not excluded [4]. The aggregation of BChl c in fluid solutions [5] and in nematic liquid crystal [6,7] has been investigated previously. It was also found [unpublished results] that BChl c in chlorosomes is photochemically stable—it cannot easily be ionized by visible light. In this work we want to check if the BChl c located in two different rigid matrices: polymethylmethacrylate (PMMA) and silica glass prepared by sol–gel method [8,9] behaves similarly to pigment molecules build into oligomers or rather to monomeric BChl c in fluid solution. Two matrices of different properties were chosen. In PMMA the covalent bonds appear whereas in silica glass oxygen occurs in two different states: part of oxygen molecules have not saturated bonds. The PMMA is a typical polymer in which spectra of several dyes and pigments were reported [10]. The hydrolysis of tetraethoxysilane (TEOS) under acidic condition yielded SiO_2 in a form of a glass-like material. The structure of this essentially anhydrous rigid matrix [8,9] is

shown in Fig. 1. The dye molecules could be enclosed inside of the formed particles or they may be bound to particle walls. The scheme of possible location of pigment molecules in such SiO_2 matrix is proposed in Fig. 1. It is interesting if in such matrix the mutual pigment interaction will be stronger or weaker than interaction with such silica walls. On the basis of literature [10], the uniform distribution of monomeric pigment in PMMA is expected.

We want to compare the aggregation and photochemical properties of BChl c in these two rigid matrices. In case of stronger interactions between pigment molecules than that with matrices applied, the pigment aggregation should be seen. In other case the monomeric pigment molecules mutually isolated by matrix have to be observed. The photostability of pigment molecules in a such two matrices could be also different.

It was shown by Krasnovsky et al. [11] that BChl c in PMMA exhibits rather intensive intercombinational radiative transition (from triplet state to ground state) seen as delayed luminescence (DL). In order to compare the efficiency of the intersystem crossing transition in both matrices we have measured DL of BChl c and bacteriopheophytin c (BPhe c) in silica glass and in PMMA in a spectral region of prompt fluorescence (α—type DL) as well as in a region of triplet–singlet transition (phosphorescence or β—type DL). The nonradiative deexcitation of the excited pigments molecules was also investigated using the photoacoustic measurements.

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● 1   ◉ 2   ○ 3   ● 4
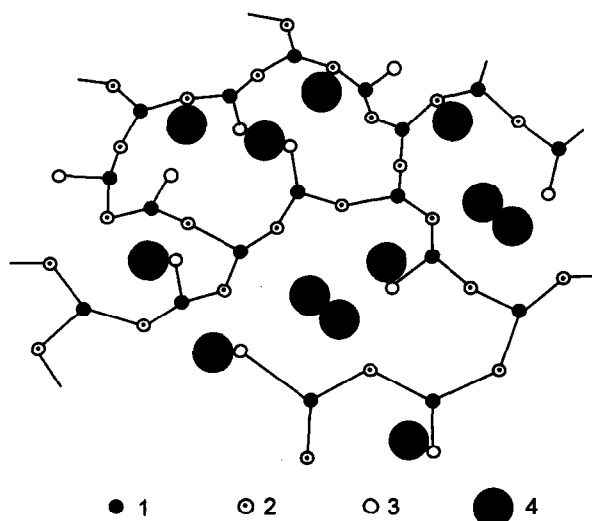
1 - Silicon atoms, 2,3 - Oxygen atoms, 4 - Pigment molecules

Fig. 1. Scheme of the structure of silica glass matrix and pigment molecules localization in this glass. Oxygen with saturated bonds is marked as 2, with unsaturated as 3.

2. Materials and methods

BChl c and BPhe c were isolated from the green sulfur bacterium *Prosthecochloris aestuarii* (strain 2 K) and thin-layer chromatographed using the method described by Dud-kowiak et al. [5]. The pigments were dissolved in chloroform. The fluid solution of PMMA with pigment was poured on the glass plate and dried during one day. TEOS (1.11 ml) was dissolved in 0.35 ml of Triton X-100 and 9.44 ml of ethanol. One drop of HCl was added. The solution was stirred on magnetic stirrer during 3 h. The pH 7 was established by the addition of NH_4 (35%). Pigment was added to neutral gel solution and mixed. The solution was poured on the glass plate and dried one or two days.

The absorption spectra were measured with a Zeiss Spe-cord M40. The steady-state fluorescence spectra were obtained using arrangement constructed in our laboratory.

The photoacoustic spectra (PAS) were measured with a single beam spectrometer constructed in our laboratory [12].

Time-resolved (in microsecond range) delayed luminescence spectra were measured with an apparatus described in detail previously [13,14]. The excitation source was a combination of a nitrogen and a dye laser (type LN120C/LD2C, PRA Laser, Canada). Pulse duration was 200 ps (full width at half maximum—FWHM). A Hamamatsu photomultiplier (model R2658P) was used in the DL apparatus. All DL spectra were taken with an additional delay of 300 ns with respect to the laser peak in order to eliminate the prompt fluorescence contributions. In order to measure spectra in vacuum at low temperatures, the sample was located in a cryostat (Closed Cycle Refrigeration Systems model TSL-22, Cryophysics, Switzerland).

3. Results and discussion

Fig. 2 shows the absorption of BChl c in PMMA and in fluid silica gel (TEOS) and in silica glass. In all three cases the spectra of samples before illumination (Fig. 2a) are characteristic for monomeric pigment. The red maximum is located about 666 nm, and the main maximum in a Soret region is observed at 434 nm. The illumination of the PMMA sample during the measurements of photoacoustic spectra result in strong spectral changes (Fig. 2b). The Soret maximum is now located at 415 nm, the red maximum at 670 nm and low but well resolved maxima at 519 nm, 552 nm and 611 nm appear (Fig. 2b). It shows the BChl c pheophytinization with the formation only one pigment form [15]. Much more complex changes are observed at the same time and degree of illumination in silica glass sample (Fig. 2b). The red band is shifted to 671 nm. It means to position characteristic for BChl c or BPhe c in nematics or in solutions with lipids [5–7]. The Soret band half band width is broader than for the same sample before illumination and also than that in a case of PMMA sample measured after illumination (Fig.
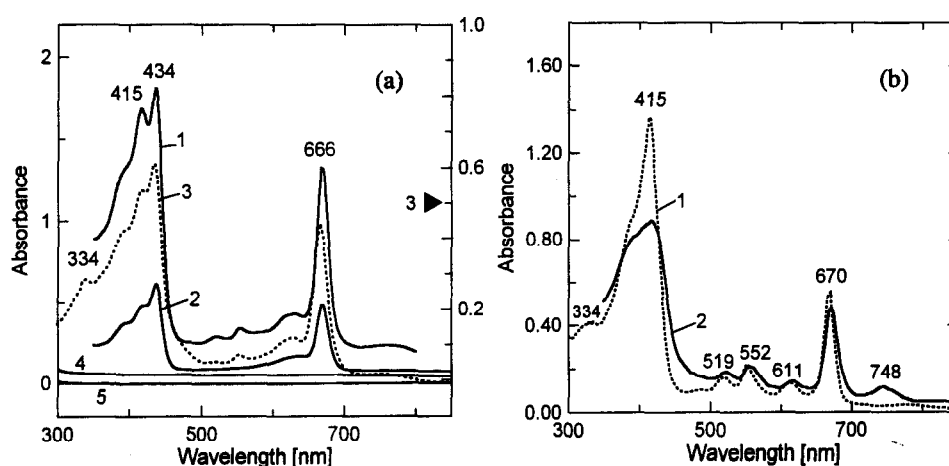

Fig. 2. Absorption spectra. (a) Samples before illumination: (1) BChl c in PMMA; (2) in fluid TEOS gel; (3) BChl c in silica glass; (4) silica glass; (5) PMMA. (b) Samples after illumination: (1) BPhe c in PMMA; (2) BChl c products in silica glass.

2b). The Soret maximum is located at 418 nm and other maxima at 523 nm, 558 nm and 618 nm are seen (Fig. 2b, curve 2) what suggest strongly at least partial pigment pheophytinization [5,6].

It is known [15,6,7] that BPhe c in chlorosomes exhibits much higher ratio of the Soret to red band than the same pigment in fluid organic solutions and than the BChl c in chlorosomes. Therefore in chlorosomes as a result of pheophytinization is usually observed not only the shift of the maximum of the Soret band but also the increase of this ratio. This increase is observed as a result of illumination in a case of BChl c in PMMA but in much lower degree for illuminated sample with BChl c in silica glass. It seems that in the rigid matrices the Bphe c absorption is similar to that in chlorosome. In a such case the higher increase of Soret to red bands ratio in a case of sample in PMMA than for a sample in silica glass suggests more efficient pheophytinization in a first than in a second case.

The maximum at 748 nm, which in unilluminated sample (Fig. 2) is seen only as a low shoulder, now increases. This maximum is characteristic for aggregated forms of BChl c and/or BPhe c. Matsuura et al. [16] had reported two major spectral forms of aggregated BChl c absorbing at 740 nm and 760 nm. The shorter wavelengths form is more susceptible to the reaction with protons. This form predominants in illuminated silica glass sample (Fig. 2). It is interesting that the concentration of this form is increasing as a result of sample illumination.

In PMMA the spectrum suggests complete pheophytinization whereas in a glass Soret band is much broader and with lower intensities ratio of the Soret to red band suggest the occurrence of the several forms of photoproducts or the BChl c + BPhe c forms. It is not excluded that BPhe c molecules located in different positions in a matrix and interacting with it and with other pigment molecules in a different degree (Fig. 1) exhibit different spectra.

Fig. 3 presents the photoacoustic spectra of both types of samples. In PMMA the BPhe c photoacoustic spectrum is rather similar to the BPhe c absorption in this matrix (Fig. 2b) whereas the PAS of illuminated sample in silica glass is much more complicated than the absorption of the same sample. The Soret band is in both cases broad, the maxima at about 520 nm, 556 nm and 618 nm are observed but low absorption maximum located at about 750 nm is now much higher with respect to red band than the same maximum in absorption. The red band is located at 670 nm. These observations suggest the occurrence of some forms of pigment exhibiting high efficiency of thermal deactivation. Probably they are same forms of BPhe c slightly only interacting with walls of silica cells. Oligomeric forms or aggregates of BChl c and BPhe c are usually absorbing at 750 nm [5–7]. Oligomers exhibit high yield of thermal deactivation. Because of the strong light intensity necessary for PAS measurements it was not possible to register the PAS of the not pheophytinized samples.
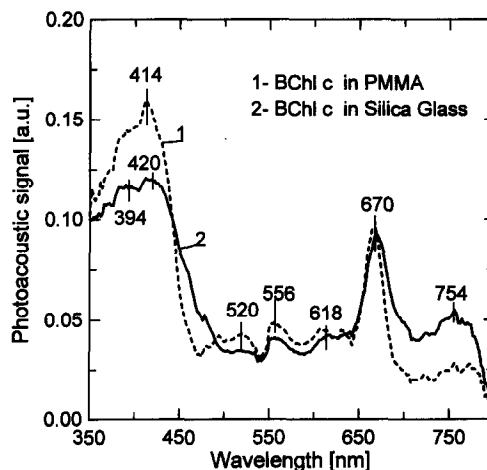

Fig. 3. Photoacoustic spectra. Samples after illumination: (1) pigment in PMMA, (2) pigment in silica glass. Frequency of light modulation 16 Hz.

Fig. 4a presents fluorescence spectra of samples. The main maximum of emission is located for both matrices at about 669 nm independently on the wavelength of excitation. It seems that only one form of pigment is efficiently fluorescent.

Fig. 4b shows the delayed luminescent spectra of samples taken in a region of emission from the excited singlet to ground state. As usually for BChl c samples in artificial matrix this emission is rather intensive and located in a region of prompt fluorescence (Fig. 4a) [7]. In a case of sample in PMMA the maximum of DL is located at 674 nm independently on the wavelengths of excitation, whereas in glass sample the DL band is broader and the maximum at excitation in a region of BPhe c Soret band (415 nm) is located at 674 nm but at excitation in a region of monomeric absorption (at 434 nm) band is shifted to 680 nm. It shows again that more than one form of pigment is contributing to DL created in the silica glass.

It was interesting to observe the radiative transition from triplet to singlet state $(T - S_0)$. It was known that in PMMA maximum of phosphorescence is located at 960 nm [11]. We have found this emission in the same spectral region (Fig. 4c). The intensity of this emission is much lower than that reported by Krasnovsky et al. [11] but it is measurable (Fig. 4c). It was shown (Fig. 4c) that this slow (50–100 μs) emission is grown as result of oxygen elimination. It is in PMMA about two times stronger without oxygen than with the presence of the oxygen (Fig. 4c). It also strongly increasing as a result of sample cooling to 8 K temperature (not show). In the silica glass at the same experimental conditions of excitation and at similar absorption of sample the measurable emission at 960 nm was not observed. It shows that in this matrix triplet states are efficiently quenched. The lower efficiency of Bchl c pheophytinization in a glass than in PMMA is due to this effect. Most of the photochemical reactions are undergoing with the participation of long living triplet states. In a case of efficient quenching of triplets the photochemical reactions became less efficient. The quench-
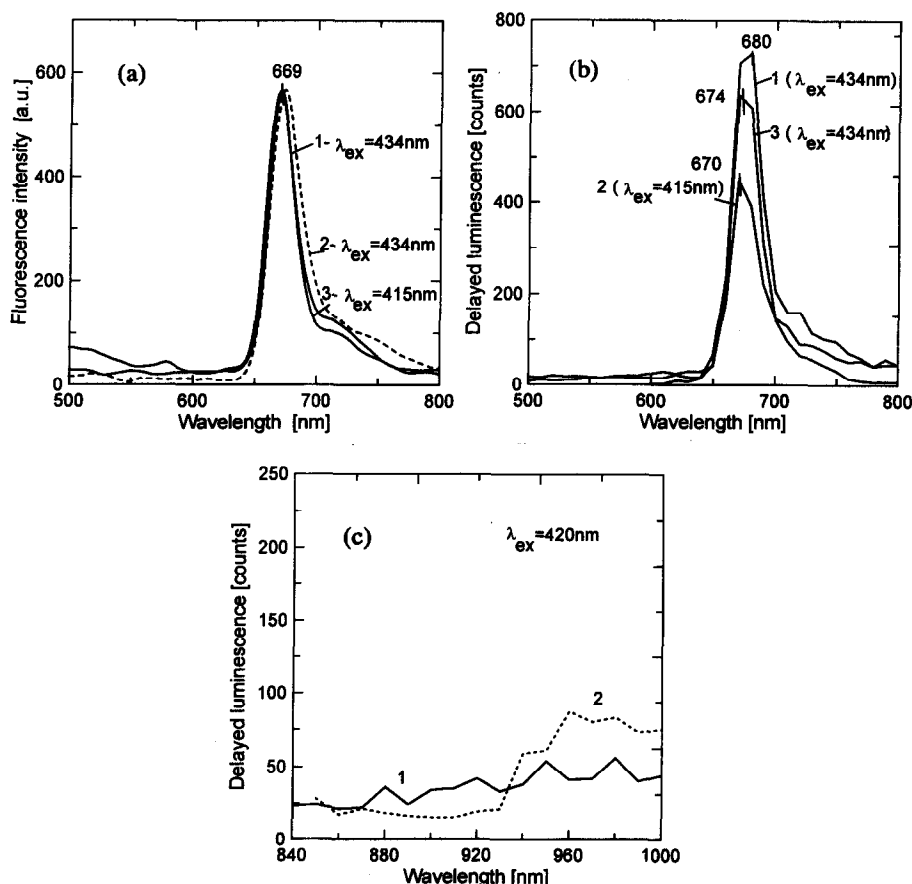
Fig. 4. (a) Fluorescence spectra of pigment: curves (1) and (3) in PMMA, (2) in silica glass. (b) Delayed luminescence spectra (α—type DL) observed in a region of prompt fluorescence. Curves (1) and (2) pigment in silica glass at two excitation wavelengths; curve (3) pigment in PMMA. Time window from 0.3 μs to 5.3 μs. (c) Phosphorescence spectra (β—type DL). Curve (1) pigment in oxygen atmosphere, (2) without oxygen. Time window: from 50.3 μs to 100.3 μs.

ing can be due to the presence nonsaturated oxygen molecules in silica glass matrix (Fig. 1).

From the comparison of the pigment spectra in two investigated matrices one can conclude that in silica glass matrix, produced by sol–gel method, BChl c is located in more similar situation to that in organisms than the pigment embedded in PMMA polymer. It is because in silica matrix pigment occurs, as in organisms, in different states of aggregation and/or with different interactions with surroundings. Pigment is also more photochemically stable in silica glass than in PMMA because of more effective quenching of triplet states in silica glass than in polymer matrix.

Comparing the pigment behaviour in investigated matrices with that in organisms we can conclude that photochemical stability depends not only on the rigidity of surroundings of pigment molecules but also on the chemical character of matrix. It is because matrix has the influence on pigment interactions with surroundings as well as with the oxygen and with other pigment molecules.

Acknowledgements

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