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Properties of bacteriochlorophyll *c* and bacteriopheophytin *c* in resting and stimulated lymphocytes

A. Dudkowiak[a], E. Staśkowiak[a], I. Hanyż[a], K. Wiktorowicz[b], J. Cofta[b], D. Frąckowiak[a,*]

[a] *Institute of Physics, Poznań University of Technology, Nieszawska 13A, 60-965 Poznań, Poland*
[b] *Department of Biology and Environmental Sciences, K. Marcinkowski University of Medical Sciences, Długa 1/2, 61-848 Poznań, Poland*

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Abstract

In order to establish the possibility of the bacteriochlorophyll *c* and bacteriopheophytin *c* applications in photodynamic therapy and diagnosis of cancer, the absorption, fluorescence emission and excitation spectra of these pigments introduced into resting and stimulated (having properties corresponding to the first stage of cancerogenesis) lymphocytes have been measured. The spectral and photochemical properties observed in stained and unstained lymphocytes are discussed. It was shown that bacteriopheophytin *c* is photochemically stable in the cells and it is to a higher degree incorporated into stimulated than resting cells; therefore it is more suitable for medical applications than bacteriochlorophyll *c*.

Keywords: Bacteriochlorophyll *c*; Bacteriopheophytin *c*; Photodynamic diagnosis; Photodynamic therapy; Resting cell; Stimulated cell

1. Introduction

Bacteriochlorophyll (BChl) *c* is a natural pigment occurring in green sulfur photosynthetic bacteria. It exhibits high optical extinction coefficient in Soret and Q bands [1,2]. Exact positions of these bands in organisms and in various solvents are different, and additionally in vivo BChl *c* occurs in highly aggregated state [3,4]. Light from the red band region can easily penetrate into the human cells [5,6]. Recently, several attempts have been made to apply the natural photosynthetic pigments in medicine [5,7–11]. Usually natural pigments are less harmful for human cells than synthetic dyes. The use of several tetrapyrrolic dyes as a second-generation photosensitizers were described [12].

In solution, the efficiency of triplet state generation of the pigments investigated has been earlier evaluated by laser induced optoacoustic spectroscopy (LIOAS) as rather high [13]. Triplet states are very effective in photodynamic reactions generation [14–18], therefore BChl *c* (Fig. 1) and its metal ion-free derivative bacteriopheophytin (BPhe) *c* seem to be promising candidates for photodynamic applications. The dye-sensitizer suitable for photodynamic therapy (PDT) or photodynamic diagnosis (PDD) has to be incorporated selectively, i.e. more efficiently into cancerous than into healthy cells. In PDT treatment, the stained cells have to be destroyed as a result of illumination. For PDD applications, the pigment in the cancerous cells has to be intensively fluorescent. The pigments studied exhibit quite a high fluorescence in solution [13], therefore, they could also be candidates for PDD applications. In both applications, the sensitizer has to be possibly non-toxic for healthy cells and quickly expelled from them.

Usually, the efficiency of the sensitizer incorporation cannot be precisely established from its absorption spectra

Abbreviations: BChl, bacteriochlorophyll; BPhe, bacteriopheophytin; LIOAS, laser induced optoacoustic spectroscopy; PBS, phosphate buffered saline; PDD, photodynamic diagnosis; PDT, photodynamic therapy; PHA, phytohemagglutinin; R, unstained resting cells; RD, stained resting cells; S, unstained stimulated cells; SD, stained stimulated cells; TD, thermal deactivation

* Corresponding author. Tel.: +48 61 665 3180; fax: +48 61 665 3178.
E-mail address: frackow@phys.put.poznan.pl (D. Frąckowiak).

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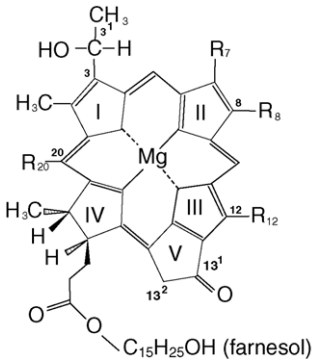

Fig. 1. Molecular structure of BChl *c* ($R_7 = R_{20}$ = methyl; R_8 = ethyl, *n*-propyl or isobutyl; R_{12} = ethyl or methyl).

because the pigments' absorption is low when compared to that of the cell material and because of the perturbations of spectra by light scattering. More reliable are emission and photothermal measurements [6,15,17,18]. In analysis of the fluorescence data one has take into account that the quantum yields of the dye fluorescence in solutions and in the cells are different [17,18]. Usually, the dye exhibiting high quantum yield of fluorescence in solvents has a lower but still measurable fluorescence quantum yield in the cells. The sequence of the fluorescence yield values measured for a set of similar dyes in solvents and in the cells is in most cases similar [17,18], therefore, some results obtained on simple models can usually be extrapolated in order to predict results in biological systems [13,16,17]. The spectral and photochemical properties of the pigments studied in solutions have been established earlier [13]. This paper reports the properties of BChl *c* and BPhe *c* in resting and stimulated lymphocytes. Stimulated cells can be used as a model for leukaemic cells [18,19]. There are several differences between lymphocytes obtained from healty donors or those artificially stimulated in vitro and leukaemic cells, but they have at least one common feature, i.e. vigorous proliferation [19].

2. Materials and methods

2.1. Pigments

The molecular structure of bacteriochlorophyll (BChl) *c* is shown in Fig. 1. Bacteriopheophytin (BPhe) *c* is an Mg-ion free derivative of BChl *c*. The culture of *Prostheochloris aestuarii* green bacteria cells, isolation and purification of BChl *c* and BPhe *c* have been described previously [20,21]. The pigments obtained were dissolved at 10^{-3} M concentration in ethanol and stored at 12 °C before use.

2.2. Isolation of the mononuclear cells

Mononuclear cells were isolated from samples of heparinized venous blood from healthy donors by centrifugation ($400 \times g$ at 4 °C for 30 min) over a Gradisol L (AQA MEDICA, Poland) gradient ($d = 1.077$ g/cm^3) according to Bøyum [22]. After centrifugation, the mononuclear cells were washed three times and resuspended to a concentration of 8×10^6 cells/ml in a phosphate buffered saline (PBS) solution.

The purity and number of mononuclear cells (monocytes and lymphocytes) were established using flow cytometry. The lymphocytes content was about 75% of mononuclear cells, therefore, the cells were henceforth described as lymphocytes. The investigated cells have some admixture of erythrocytes attached to lymphocytes as it can be seen from the absorption spectra (Fig. 2B).

2.3. Cell stimulation and incubation with pigments

One portion of lymphocytes (a concentration of 8×10^6 cells/ml) was stimulated with 10 μg/ml phytohemagglutinin (PHA, HA 17, Wellcome, England) for 1 h at 37 °C. After the incubation, the cells were washed twice and resuspended in a PBS solution (concentration 40×10^6 cells/ml). The unstimulated (resting) cells were prepared in a similar manner but were not incubated with PHA. Next, 1 μl of ethanol pigments solution (10^{-3} M) per 1 ml cells was added to samples of both stimulated and resting cells. The control samples were cells with addition of the same volume of ethanol. The time and condition of incubation e.g. concentration were established on the basis of previous our results [23]. The incubation of the cells with pigments lasted for 1 h (37 °C) in the dark. After the incubation, the cells were washed three times with a PBS solution.

The amount of pigments incorporated into lymphocytes was evaluated taking into account the absorbance of cell suspension (measured at absorption maximum of pigment monomer) in a PBS solution and amount of cells in the sample (counted using flow cytometry) as well as the volume of all cells in the sample. From the measurements it follows that the average concentration of BPhe *c* in the stimulated cell suspension was about 1.08×10^{-6} M whereas the BPhe *c* concentration calculated inside the stimulated cell was about 0.81×10^{-4} M. The concentration ratio of this pigment incorporated into stimulated and resting cells was about 1.93. The evaluation of number of all BChl *c* molecules absorbed per cell is more complicated because of its aggregation.

The lymphocyte samples used were differentiated as follows: resting (R) and stimulated (S) unstained cells, resting (RD) and stimulated (SD) cells stained by pigment. The type of sensitizer is marked in brackets, e.g. RD(BChl) or RD(BPhe), which denotes the resting cells stained by BChl *c* or BPhe *c*, respectively.

2.4. Spectral measurements

The absorption, fluorescence emission and fluorescence excitation spectra were measured, using Specord M40 (Carl Zeiss, Jena, Germany) and Fluorescence Spectrophotometer

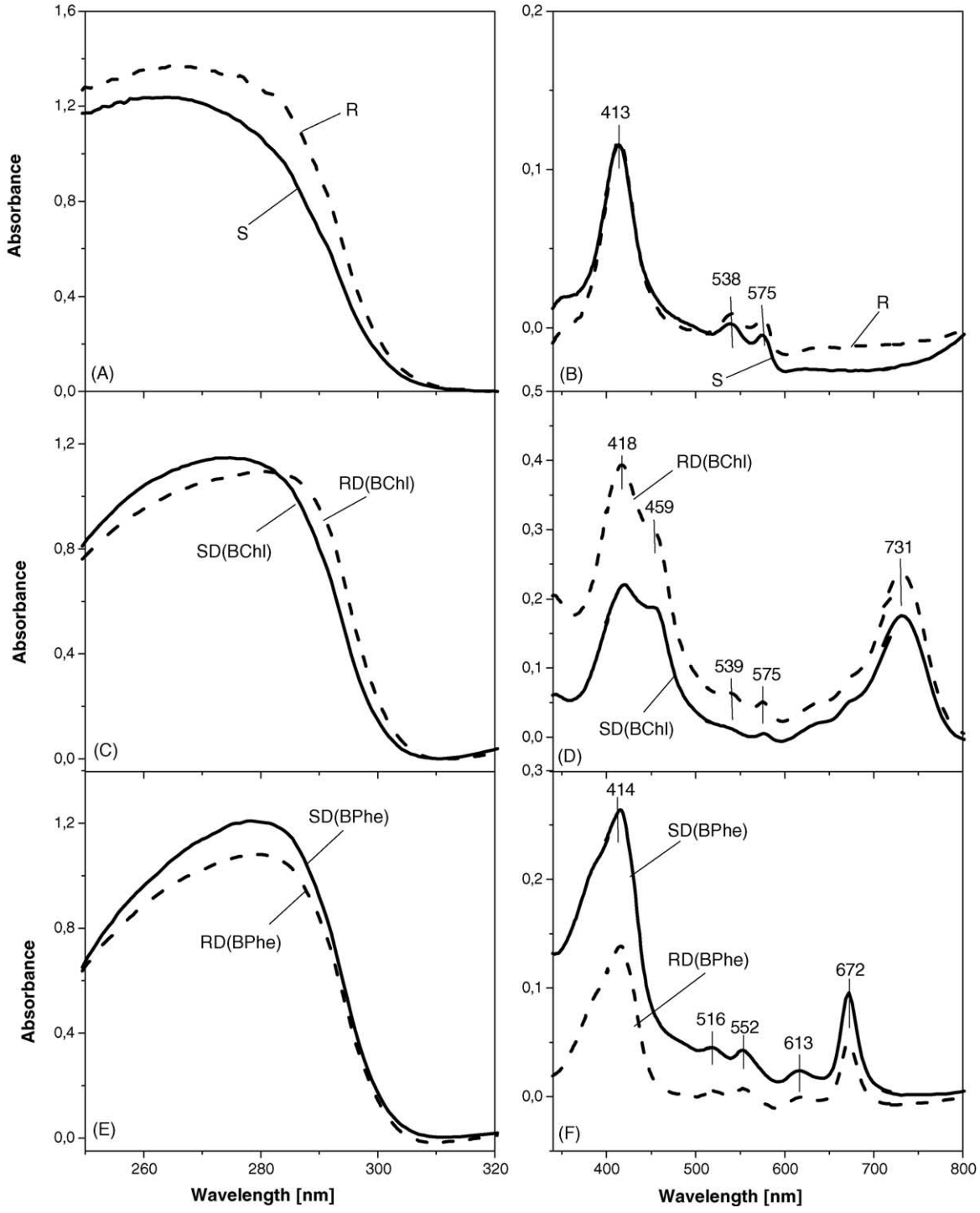

Fig. 2. Absorption spectra of resting and stimulated lymphocytes stained by BChl *c* (A, B) and BPhe *c* (C, D); description of curves: resting (RD) and stimulated (SD) stained by pigment-sensitizer cells; type of pigment was marked in brackets.

F4500 (Hitachi, Tokyo, Japan), respectively. The same samples were used for the measurements of the absorption as well as fluorescence emission and excitation spectra. The samples were located in 1 cm quartz square cuvettes. The fluorescence spectra were recorded at right angle observation of the center of a centrally illuminated cuvette. The influence of fluorescence attenuation due to the absorption of exciting beam as well as reabsorption of emission and its secondary fluorescence was evaluated on the basis of formula:

$$F_{\mathrm{corr}} = F_{\mathrm{obs}}\,\mathrm{antilog}[(\mathrm{OD}_{\mathrm{exc}} + \mathrm{OD}_{\mathrm{em}})/2] \quad (1)$$

where F_{obs}, F_{corr} are the intensities of observed and corrected fluorescence emission, respectively; $\mathrm{OD}_{\mathrm{exc}}$ and $\mathrm{OD}_{\mathrm{em}}$ the optical densities of sample at excitation wavelength and at

maximum of emission band, respectively [24]. The differences between F_{corr} and F_{obs} were estimated to be about 15–20% for monomeric forms of BPhe *c*. The aggregation of BPhe *c* was not observed in the investigated systems. In a case of BChl *c*, the absorption and emission spectra of monomers and aggregates are superimposed [2,4,20,25], therefore the introduction of such correction is much more complicated. In a such complex situation, the F_{obs} values were used, in further discussion. In applied approximation, the ratio of observed monomeric emissions of the same pigment in two types of cells (R and S) can be compared, because the fluorescence yields of monomeric form of pigments located into R and S cells are similar, as it follows from the comparison of absorption and fluorescence excitation spectra.

The photochemical reactions were induced by semiconducting laser SN-20899167 (Optel, Opole, Poland). This laser emits the light with a 638.2 nm wavelength giving the intensity of the illumination equal to 7.6 mW/cm^2. The dose of the light energy that reached the samples in 1 h was about 27.4 J/cm^2, which was almost the same as that used in [26] for killing 90% of cancer cells.

3. Results

Fig. 2 shows the absorption spectra of the unstained cells (Fig. 2A and B) and the cells stained by BChl *c* (Fig. 2C and D) and by BPhe *c* (Fig. 2E and F). The absorption due predominantly to the cell material (240–320 nm, Fig. 2A, C and E) is much higher than that in the region of hem and the added sensitizer absorption (350–800 nm, Fig. 2B, D and F). For stained cells, the short-wavelength absorption of resting and stimulated cells is similar (Fig. 2C and E), which means that the pigment incorporated into the cells does not disturb nor change strongly the cell material absorbing in this spectral region. The tryptophan has a absorption maximum in the range of 289–293 nm, whereas DNA, RNA, guanine, cysteine and several other compounds exhibit an absorption in the range of 250–270 nm [24]. It seems that as a result of the cell stimulation and staining, the absorption of various cell compounds in resting and stimulated cells changes in a different way. From the difference absorption spectra (RD–R) and (SD–S) (not shown) it follows that the changes in the short-wavelengths region have similar character for both pigments. The tryptophan absorption in the stimulated cells decreases as a result of cell staining, whereas the contributions to its absorption in this region are even increasing in the stained resting cells. The increase or decrease in the absorption of some cell compounds observed as a result of cell staining can be due to the degradation of some more complex species into smaller elements or/and to a change in the intermolecular interactions between some parts of the cell material.

The spectra of unstained cells in the long-wavelengths range of absorption exhibit the maxima of hem at 413, 538 and 575 nm (Fig. 2B). The addition of a sensitizer results in

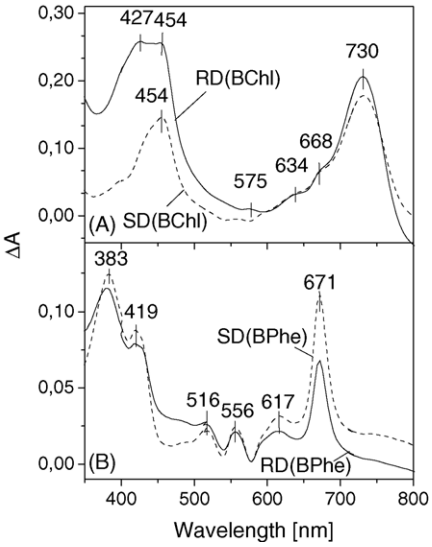

Fig. 3. The difference between absorption of stained and unstained cells (ΔA) for resting and stimulated lymphocytes; the cells were stained by BChl *c* (A) and by BPhe *c* (B); description of curves as in Fig. 2.

the appearance of additional absorption bands in the long-wavelength region especially well seen in the region of BChl *c* Q_y band at 731 nm, and the analogous band of BPhe *c* at 672 nm (Fig. 2D and F) [4,25,27]. It is known [2,4,20,25] that the formation of BChl *c* aggregates is reflected by a large red shift of its Q_y transition with respect to that of the monomeric form located at about 670 nm. In Soret band because of overlapping absorption of hem it is not easy to separate the contribution from aggregated form of BChl *c*. The maximum at 459 nm suggests the occurrence of such forms. Assuming that the extinction coefficient of pigment does not change so much from resting to stained lymphocytes and is approximately the same for photosensitizer into RD and SD cells, as follows from Fig. 2D, more BChl *c* molecules have been introduced with RD(BChl) than with SD(BChl) cells. It is of course an undesirable effect from the point of medical applications. The total BChl *c* absorption (Fig. 2D) is even slightly higher than that of BPhe *c* (Fig. 2F). More suitable for the medical application seems to be the behavior observed for BPhe *c*, which shows higher absorption of the sensitizer in SD(BPhe) than in RD(BPhe) cells (Fig. 2F). This is clearly seen not only from the Q_y region but also from the increase in the absorption of the Soret band. It should be also noted that BChl *c*, as follows from literature [2,4,20,25] and the absorption spectra (Fig. 2D), incorporated into cells mainly in the aggregated form, whereas BPhe *c* (Fig. 2F) predominantly in the monomeric form.

Fig. 3 presents the difference in the absorption spectra obtained by the substraction of the stained and unstained cells absorption for BChl *c* (Fig. 3A) and BPhe *c* (Fig. 3B). From the difference absorption spectra it follows again that BPhe is more effectively incorporated into the stimulated than into the resting cells, i.e. this pigment has higher absorbance in SD than RD cells (Fig. 3B). The maxima at 516, 556 and 617 nm

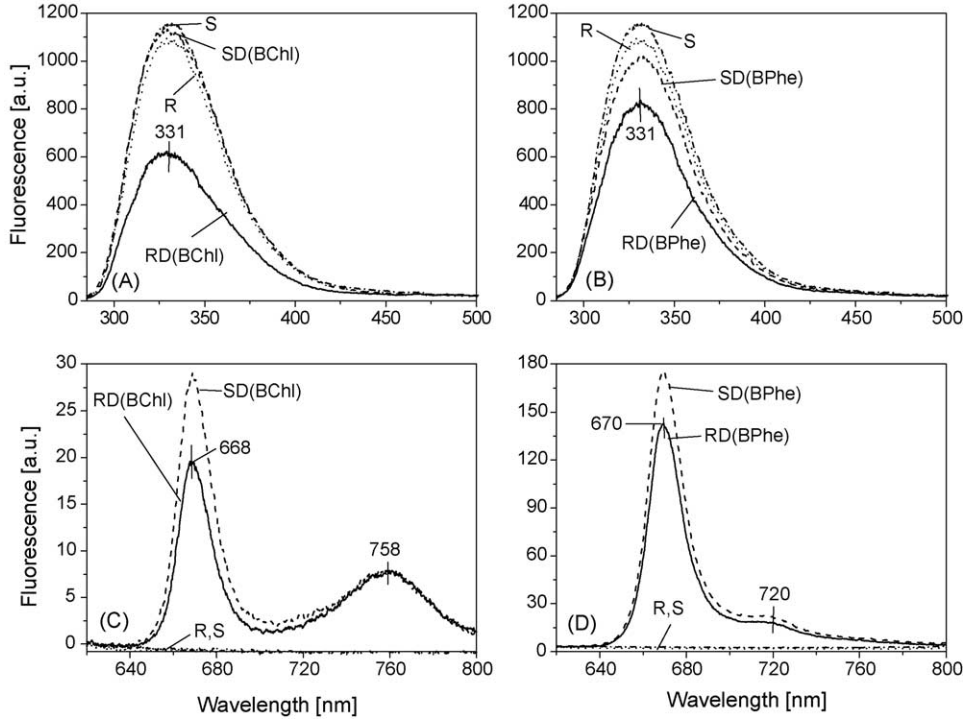

Fig. 4. Fluorescence spectra of lymphocytes unstained and stained by BChl *c* (A, C) and BPhe *c* (B, D); description of curves as in Fig. 2; wavelength of excitation λ_{exc}: (A, B) 275 nm; (C) 434 nm (at maximum of BChl *c* monomer absorption) and (D) 414 nm (at maximum of BPhe *c* monomer absorption).

appearing in the absorption difference spectra (Fig. 3B) are related to the vibrational and $Q_x(0,0)$ transitions of BPhe *c* [20]. BChl *c*, as follows from Fig. 3A, exists mainly in aggregated form, and it is to a higher degree incorporated into and/or attached to the resting than the stimulated cells.

Fig. 4 shows the fluorescence spectra of samples excited in the region of predominant absorption of the cell material (Fig. 4A and B) and in the region of pigment-sensitizer absorption (Fig. 4C and D). Fig. 4A and B shows that the fluorescence intensity of the cell material of the unstained resting (R) and stimulated (S) cells is similar. The fluorescence of the cell material observed for RD and SD cells shows much different behavior than that of the cell material absorption of these samples (Fig. 2C and E). It shows that in both type of cells, the cell staining changes the fluorescence yield of the cell material more strongly in the resting than in the stimulated cells (Fig. 4A and B). The shapes of the fluorescence spectra in the 620–800 nm region (Fig. 4C and D) suggest once more a different degree of aggregation of BChl *c* and BPhe *c* incorporated into both types of the cells [4,20,25]. It is seen (Fig. 4C) that BChl *c* exists in monomeric and aggregated form characterized by the emission at 668 nm and at about 758 nm, respectively, while the fluorescence spectra of BPhe *c* (Fig. 4D) indicated the presence of monomers showing emission at 670 nm with shoulder at 720 nm. The ratio of the emission due to the aggregated form to that assigned predominantly to monomers in the cells stained by BChl *c* is higher for the resting lymphocytes than for the stimulated cells (ratio of maximum at 758 nm to that at 668 nm). It suggests different interactions of these pigment forms with the membrane and/or with the material compounds in the stimulated and resting cells. Intensity of the emission of aggregates of BChl *c* is similar in resting and stimulated cells, whereas in the range of the emission of pigment monomers (at 668 nm) the emission of SD cells is higher. The emission of aggregates can be due mainly to excitation energy transfer from excited monomeric forms of pigment and it seems that aggregates are incorporated and/or may be attached to the cell membrane to a similar degree into both types of cells. BPhe *c* rather does not aggregate in the cells (Fig. 4D) and it is introduced more efficiently into the SD than into the RD cells. It is in agreement with the absorption results (Figs. 2F and 3B). The resting cells stained by BChl *c* exhibit higher absorption than stimulated cells (Figs. 2D and 3A), whereas the fluorescence of SD(BChl) cells is higher than that of RD(BChl) (Fig. 4C). This sequence is caused by various fluorescence yields of BChl *c* forms occurring in these two types of cells. This shows again that it is risky to draw information about dye incorporation only on the basis of the absorption or emission spectra.

Fig. 5 presents the fluorescence excitation spectra of the cells stained by BChl *c* (Fig. 5A–D) and by BPhe *c* (Fig. 5E and F). The emission was observed at 690 nm (Fig. 5A, B, E and F), i.e. in the region of the pigments' monomer emission and at 758 nm (Fig. 5C and D), i.e. in the region of the BChl aggregate emission. As follows from Fig. 5A, C and E, the energy absorbed by the other compounds of the cell material is transferred to tryptophan which contributes predominantly

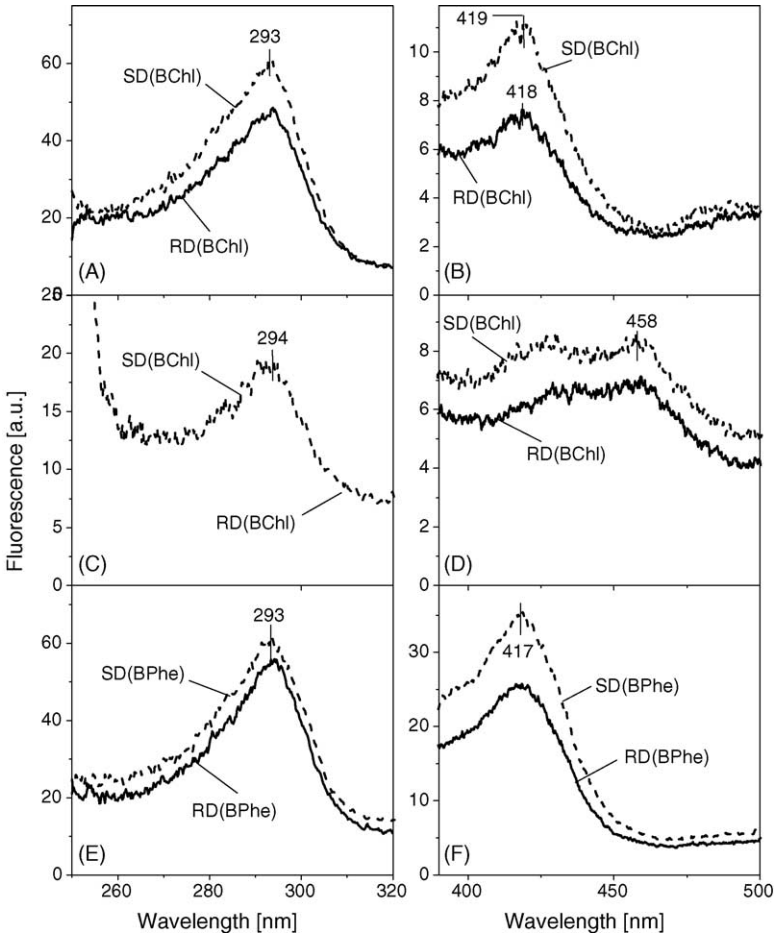

Fig. 5. Fluorescence excitation spectra of lymphocytes unstained and stained by BChl *c* (A–D) and BPhe *c* (E, F); description of curves as in Fig. 2; wavelengths of observation λ_{obs}: (A, B, E, F) 690 nm (at monomeric forms of pigments' emission) and (C, D) 758 nm (at aggregated form of BChl *c* emission).

to the pigment emission. It is known [28] that the energy absorbed by tyrosine is efficiently transferred to tryptophan or to its complex [29,30]. In the region of the pigment absorption (Fig. 2B) and emission (Fig. 4C and D), the spectra of the unstained cells exhibit also very low contributions from the cell material. In the 390–500 nm wavelength region (Fig. 5B, D and F) the emission of the cells stained by BPhe *c* is higher than that of the cells with BChl *c*, and for both pigments the emission from the stimulated cells is more intensive than that from the resting cells. This effect is similar to that observed for the emission spectra (Fig. 4C and D). For the cells stained by both sensitizers, at excitation in the Soret band range, the contributions to the fluorescence observed at 690 nm (Fig. 5B and F) are higher for the stimulated cells than for the resting cells. The shape of the fluorescence excitation spectrum of BChl *c* observed at 758 nm (Fig. 5D) is of course different than that recorded at 690 nm emission (Fig. 5B). The maximum at 458 nm (Fig. 5D) strongly suggests the involvement of the aggregated forms in the emission at 758 nm.

Fig. 6 shows the fluorescence excitation spectra observed at the cell material emission (at 330 nm observation) excited in the range of 250–320 nm. The results for unstained cells as well as for stained cells by both sensitizers are approximately similar. The small differences in spectral properties for stained cells observed between Figs. 5A, C, E and 6 could be related to different efficiencies of the excitation energy transfer between various components. The ratio of fluorescence excitation spectrum divided by absorption, both measured in cell material absorption wavelength region, exhibit in the short-wavelength part a practically constant value increasing in a region of tryptophan absorption. It suggests that a part of the excitation energy absorbed by tryptophan is emitted as its fluorescence (Fig. 4A and B) and the light absorbed by other components of cell material is partially transferred to tryptophan or its complexes. Emission of tryptophan complexes and oxidation products is lately intensively investigated [29,30].

The changes in the relative fluorescence occurring as a result of illumination were calculated as F/F_0, where F_0 is the fluorescence before illumination, F the fluorescence observed for various times of illumination. The graphs in Figs. 7 and 8 illustrate the kinetics of the fluorescence intensity changes due to illumination. Fig. 7A, presenting the fluorescence of the cell material (λ_{exc} = 275 nm), implies that the resting cells stained by BChl are destructed more quickly than the stimulated cells. According to the data in Fig. 2D, there are more

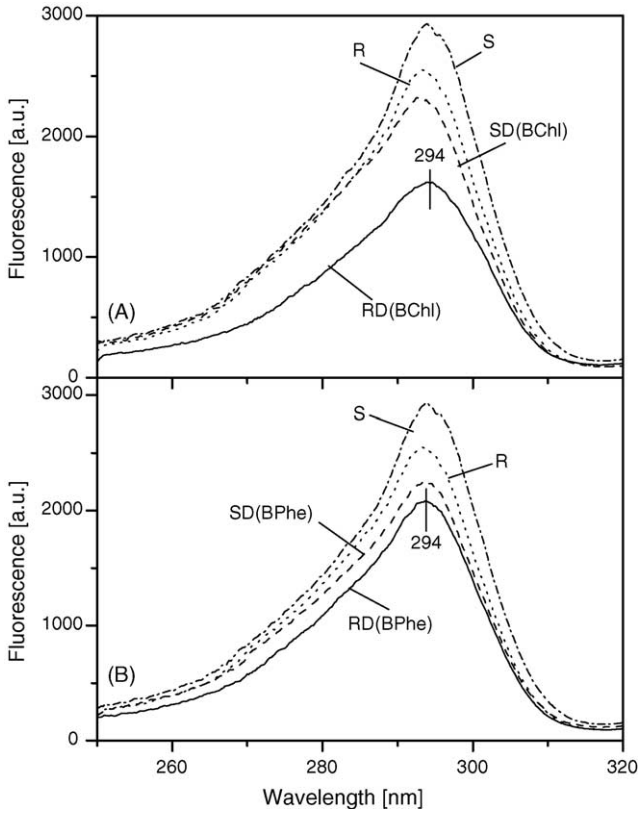

Fig. 6. Fluorescence excitation spectra of lymphocytes unstained and stained by BChl *c* (A) and by BPhe *c* (B); wavelength of observation λ_{obs}: 330 nm (tryptophan emission); description of curves as in Fig. 2.

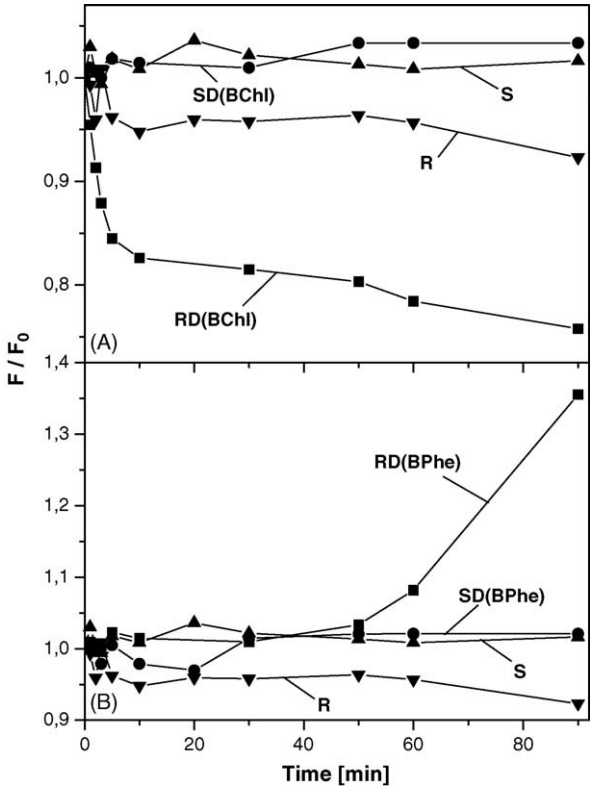

Fig. 7. Changes in normalized fluorescence intensity (measured at 330 nm) as a function of time of illumination for unstained and stained by BChl *c* (A) and by BPhe *c* (B) lymphocytes; F_0, fluorescence intensity before illumination; *F*, fluorescence intensity after various times of illumination; wavelength of excitation λ_{exc}: 275 nm.

aggregated forms of BChl *c* interact with RD than with SD cells. The presence of BChl *c* into and/or in close proximity of the cells generates changes in the cell material fluorescence. Unstained resting and stimulated cells are rather stable under similar illumination (Fig. 7A). As follows from Fig. 7A, BChl *c* cannot be applied as a sensitizer in PDT. Fig. 7B shows that on exposure to the same illumination conditions the stimulated cells stained by BPhe are stable. The increase in the fluorescence of RD(BPhe), excited at 275 nm, on illumination for longer times may be due to photoproduction and/or to photoinduced alteration of some fluorescent cell compounds. It suggests the occurrence of some effects and products related to the illumination of RD cells. The same conclusions, as from Fig. 7, follow from the changes in the fluorescence excitation spectra observed in the region of the cell material absorption near the tryptophan emission maximum at 330 nm (not shown). Similar kinetics of the relative fluorescence changes can be observed, which suggest that the amount of the destroyed material is related to the amount of introduced pigments and/or time of illumination.

Fig. 8 presents changes in the emission of the pigments introduced into cells. It shows that the unstained cells are stable as their emission in the region of the pigments fluorescence does not change. As a result of illumination, the BChl *c* monomer emission (λ_{exc} = 434 nm) in stimulated cells decreases and in the resting cells slightly increases (Fig. 8A). The increase (Fig. 8A) is due to the pigment disaggregation because parallel is observed the decrease in the long-wavelength emission at 758 nm (Fig. 8B). But in SD cells, the decrease in the fluorescence intensity at both monomer and aggregate maxima (at 690 and 758 nm) testifies to the BChl *c* bleaching (Fig. 8A and B). For BPhe *c* (Fig. 8C), the bleaching is not observed, which means that this pigment is able to take part in the photodynamic action even on a long time illumination. The increase in fluorescence of BPhe *c*, excited at 414 nm, in SD and RD cells cannot be due to disaggregation, because this pigment occurs in the cells predominantly in the monomeric form (Figs. 3B and 4D). This increase can be explained by changes in the BPhe *c* interactions with the surroundings or/and the photogeneration of some strongly fluorescent products of this pigment degradation [31]. The fluorescence intensity changes due to illumination provide the information on the kinetics of the photochemical reactions or/and on possible changes in the amount and properties of the fluorescent material occurring during the illumination. It was shown [29,30] that photochemical and photobiophysical changes of biological complexes can be a source of the decrease or of the increase of their emission. The kinetics presented in Figs. 7 and 8, suggest that the sensitizer is not expelled from the resting cells in the illumination time. The amount of BPhe incorporated into stimulated cells is higher

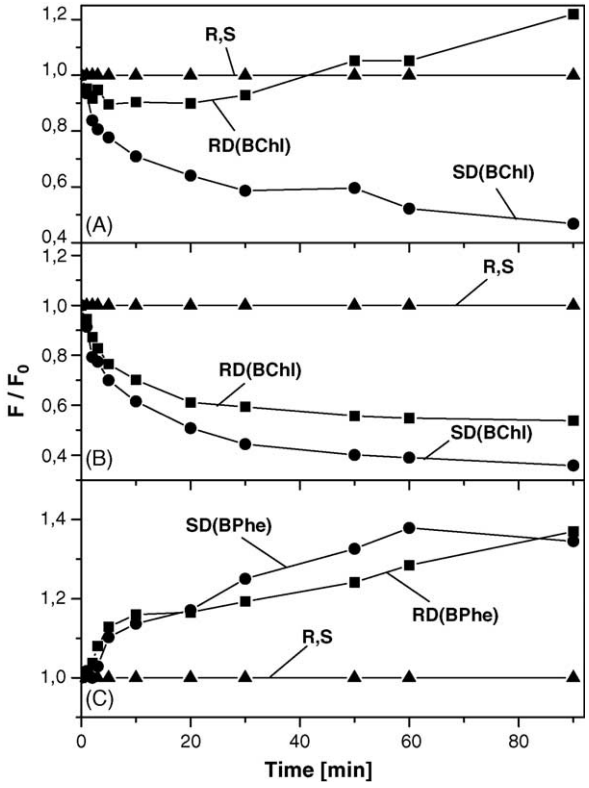

Fig. 8. Changes in normalized fluorescence intensity as a function of illumination time for stained by BChl *c* (A, B) and by BPhe *c* (C) lymphocytes; wavelengths of excitation λ_{exc}: (A, B) 434 nm, (C) 414 nm; wavelengths of observed fluorescence λ_{obs}: (A, C) 690 nm, (B) 758 nm; F_0, fluorescence intensity before illumination; *F*, fluorescence intensity after given time of illumination.

than in resting cells, therefore, the amount of the destructed cell material should be greater in the former cells.

4. Discussion

BChl *c* and BPhe *c* have been reported [13] to exhibit efficient generation of triplet states in solution, therefore, they have been supposed to be efficiently involved in photochemical reactions. The results obtained indicate that in the applied conditions of the cell incubation the photodynamic reactions have not been very efficient and in some cases their kinetics has been the same for the resting and the stimulated cells. It suggests that the pigment-sensitizer molecules are still present in the resting cells. The BChl *c* molecules are not photochemically stable in the cells and are introduced to a higher degree into resting than into stimulated cells. Additionally, the aggregation effect influences the process of BChl *c* introduction into cells. A more promising candidate for PDT application is BPhe *c*, which is in monomeric form and to a higher degree incorporated into the stimulated than into the resting cells as well as it is more stable in the cells than BChl *c*. It is known [32] that the monomers of the pigments investigated efficiently produce singlet oxygen. Besides, BPhe *c* exhibits a relatively high yield of fluorescence [13], so can be also used in PDD.

Molecular interpretation of the results presented is not easy at this stage of investigation. The uptake of the sensitizers by cancer cells and their clearance rate from healthy cells depends on their hydrophobicity or hydrophylicity [5,20,25,27,33,34]. Hydrophobicity enhances the pigment inclusion into the transformed cells but slows down the pigment clearance from normal cells [5]. BChl *c* and BPhe *c* are amphiphilic molecules, therefore, it is not easy to predict the efficiency of their incorporation into cells and their clearance from normal cells.

BChl *c* and BPhe *c* in solutions have high optical extinction coefficient in the region of low cell material absorption and they exhibit high yield of the triplet states generation [13]. Therefore, from this point of view, both dyes seem to be good candidates for PDT applications, but each of these pigments is a mixture of several forms [20,21,25] which can have different properties after incorporation into cells. As follows from our results, BChl *c* is not a promising candidate for PDT and PDD. Much better properties for these applications exhibits BPhe *c*. Several water soluble chlorophyll-type pigments derivatives have shown promising properties for medical application [5,7,8]. The singlet oxygen generation and effective photodynamic action depend on the side groups attached to the pigment chlorine-ring and the metal ion introduced in the center of the ring [9–11,27,33–35]. Therefore, in future we intend to investigate several chemically modified, water soluble derivatives of BChl *c* and BPhe *c*. On the basis of the presented results, BPhe *c* seems to be better candidate for PDT and PDD, but before the practical application further investigation, which are now in progress in our laboratory, are necessary, e.g. measurements of triplet state generation for pigment incorporated into the cells.

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