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Interactions between chlorophyll *a* and β-carotene in nematic liquid crystals

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Abstract

Fluorescence lifetime in the ps range, polarized absorption, polarized fluorescence spectra and delayed luminescence time resolved spectra were measured for chlorophyll *a* solutions with and without β-carotene addition in nematic liquid crystal. Photoacoustic spectra of the same samples at various frequencies of light modulation were also taken. The frequency dependence of the photoacoustic spectra suggests that part of the excitation is converted into heat in a slow process (with a decay time of the order of ms). The lifetime results suggest that at used concentration some aggregation of the chlorophyll *a* occurs. The chlorophyll *a* molecules interact strongly with the β-carotene forming some nonfluorescent or weakly fluorescent aggregates characterized by having various thermal deactivation yields and orientations in anisotropic matrix when compared to those of separated pigments. It seems that the aggregated forms of the chlorophylls are partially disrupted as a result of the their interaction with the β-carotene. Singlet excitation of β-carotene is not transferred to the fluorescent form of chlorophyll *a*. Delayed (in μs time range) luminescence of chlorophyll *a* is quenched by β-carotene. This luminescence is located in the same spectral region as prompt fluorescence. Interactions between chlorophyll *a* and β-carotene depend on the degree of pigment orientation and their aggregation.

Keywords: β-Carotene; Chlorophyll *a*; Excitation energy transfer; Fluorescence; Nematic liquid crystal; Photoacoustic spectra; Polarized light

1. Introduction

Several anisotropic model systems in which the molecules are oriented have been used in order to establish the interactions between photosynthetic pigments at known mutual orientations [1–3].

One such type of model of a biological membrane

Abbreviations: DL: Delayed luminescence; Chl: Chlorophyll; ET: Excitation energy transfer; H: Horizontal; LC: Nematic liquid crystal; DL: Linear dichroism; PAS: Photoacoustic spectra; Photoacoustic signal; TD: Thermal deactivation; TM: Transition moment; V: Vertical

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can be formed by the layer of oriented nematic liquid crystal (LC). Such a medium is able to orient pigment molecules embedded in it by the 'guest–host' effect [4,5]. Therefore several effects such as the influence of external electric [6,7] and magnetic [8–10] fields, and the quenching of fluorescence by oriented quenchers [10,11] have been measured for chlorophylls embedded in liquid crystal cells. The interactions between chlorophyll (Chl) *a* and Chl *b* have also been investigated [12]. In the present paper, we report on an investigation of the interaction between Chl *a* and β-carotene in nematic liquid crystal cells. Carotenoids in living photosynthetic organisms work as antenna pigments [13], but the singlet excitation of carotenoids can only be efficiently transferred to Chl *a* at the proper mutual pigment orientation and when they are in close proximity with each other [14–16]. Such conditions are fulfilled in photosynthetic complexes [17–21]. The Chl *a* fluorescence can easily be observed, but the carotenoid emission is weak [22], which means that the excitation energy transfer (ET) between the carotenoids and the Chl is usually observed as the sensitization of Chl emission [14–16] or by a change of the thermal dissipation of the energy absorbed by the carotenoids in the presence of Chl [18–21]. In addition to the singlet–singlet interactions between the carotenoids and the chlorophylls there is also a very effective quenching of the Chl triplet excitation by the carotenoids. This effect is responsible for the protective action of the carotenoids against chlorophyll photooxidation. Both effects can be investigated in organisms and in model systems. In the present paper we have measured polarized fluorescence, lifetime (τ) of fluorescence, delayed luminescence (DL), absorption and photoacoustic spectra (PAS) of Chl *a*, β-carotene and their mixture in nematic liquid crystal cells. The very strong interaction between the Chl and the LC molecules prevents the formation of the large oligomers previously observed in the hydrated polymer matrix [23–25]. The 'guest–host' effect causes the orientation of the Chl and the β-carotene. Therefore the molecules of both pigments can be located close together with definite mutual orientations and it should be possible to fulfil the condition necessary for the effective transfer of the singlet excitation of carotene to Chl, similar to that which is observed in vivo.

2. Materials and methods

The Chl *a* was purified chromatographically according to the method described in [26]. The β-carotene (Fluka AG, Chem. Fabrik CH-9470 Buchs) was used without further purification. Pigments were dissolved in a nematic liquid crystal mixture of *p*-methoxybenzylidieno-*p*-butylaniline (MBBA) + *p*-ethoxybenzylidieno-*p*-butylaniline (EBBA) (3:2). Both liquid crystals (from E. Merck, Darmstadt) were used without purification. Chlorophyll *a* was readily soluble in the LC used [4,27]. It was shown from resonance Raman spectra [28] that in the LC the Mg atom of Chl *a* is pentacoordinated. It forms ligands with the LC molecule or/and with traces of water. The 9-ketone groups are free from intermolecular bonding. The oligomers formed by Mg and ketone groups are absent. At high (10^{-2} M) concentrations some 'dry' aggregates [29–31] of Chl *a* (formed without water participation) were observed [12]. The samples with β-carotene in LC contain a different concentration ratio of monodispersed and microcrystalic forms depending on the method of the preparation of the solution. Two methods of β-carotene solution preparation were used. In both of them the concentrated (10^{-2} M) β-carotene solution was prepared first by introducing the pigment to LC using chloroform solution and evaporating the chloroform. In the first method this solution was diluted using only the LC up to reaching final concentration (usually 5×10^{-3} M). This solution was not stable in the beginning — the concentration of microcrystals increases and as a result the β-carotene absorption decreases and the light scattering on the sample increases. After some hours after preparation, the equilibrium of both forms were reached (the concentration ratio of monodispersed to microcrystal forms was low). Such a sample was stable for several weeks. In the second method a dilution of concentrated solution was prepared using a chloroform and LC mixture and then the chloroform was evaporated. The final concentration of pigment was as in the first method, but the monodispersed pigment form predominates (as it follows from a comparison of the amount of introduced pigment with that evaluated from the absorption spectrum). This sample was also stable for a long time. As it is well known that the aggregation of carotenoids enhances the interaction

between these pigments and chlorophylls [32] and that carotenoids are usually in microcrystalic form in monolayers [33], the interaction with Chl *a* of both types of β-carotene in LC preparations were compared.

The investigated solution was located in the measuring cell with the windows from the conducting glass covered by an 'orienting' layer. Two methods of preparing the orienting layers were used: (1) the technique of rubbing the polyamid layer [4] and (2) by *vacuum* deposition of the SiO_x layer [7]. The first method produces cells in which pigments have a low degree of axial orientation, the second one enables a high linear dichroism of chlorophyll absorption to be reached. In both cases photoselection by polarized light gives the polarized fluorescence spectra. Details of the liquid crystal cell construction are given elsewhere [34,35]. In some of the measurements the same solutions were used located between glass plates without an orienting layer. The investigated sets of samples differ in pigment concentrations, degree of carotene crystalization and cell window preparations. The notations of the sets of samples used in the text and in the figures captions are given in Table 1. The absorption spectra were measured with a Zeus Specord M40. The fluorescence, fluorescence excitation spectra and delayed luminescence spectra were recorded with the arrangements constructed in Poznań. All arrangements were equipped with special sample holders and polarizers and were connected to an on-line computer. The fluorescence excitation spectra have been corrected for light excitation energy.

Time resolved (in μs time ranges) delayed luminescence (DL) spectra were obtained with an arrangement which has been described in detail previously [36]. The excitation in the DL apparatus was a nitrogen and dye laser (type LN120C/LD2C, PRA Laser, Canada). The pulse duration was about 200 ps (FWHM). The intensity at the excitation wavelengths was sufficiently low to avoid nonlinear effects. An Hamamatsu photomultiplier was used in both the fluorescence and the DL apparatus.

The fluorescence lifetimes of the Chl *a* and the Chl *a* with β-carotene solutions in the nematic liquid crystals were measured using a pico and nanosecond time correlated single photon counting fluorometer built at the Center for Fluorescence Spectroscopy, School of Medicine, University of Maryland at Baltimore, MD, USA. The impulse response of this instrument was 50 ps full width at maximum (FWHM) with an MCP-PMT Hamamatsu R2809 red-sensitive detector. The source of exciting light was an Nd:YAG mode-locked laser (2 W at 532 nm; FWHM = 100 ps) and a Piridin 1 cavity-dumped dye laser. The frequency repetition rate was 1 MHz. The excitation wavelengths were 380 and 670 nm with a pulse width of 5 ps. The excitation line 532 nm was also used. The fluorescence emission was measured at 680 nm ±5 nm using either a band-pass interference filter (Ditric Optics, Inc., Hudson, MA) or broad band filters: RG-715 and RG-780 (Androver Corp., Salem, NH). Data were analyzed by summing discrete exponentials using IBH time–domain software (IBH Consultant, Ltd.).

The PAS spectra were measured with a single-beam spectrometer [37] constructed at Poznań University of Technology. PAS spectra at various frequencies of light modulation were measured [38] to distinguish between slow thermal deactivation (TD) from metastable states or traps and quick, nonradiative transitions. The value of $\nu\tau$ (where ν is the frequency of light modulation and τ the nonradiative relaxation time) influences the phase shift between

Table 1
Notation of investigated sets of samples

Set notation	$c_{\text{Chl } a} \cdot 10^{-3}$ [M/l]	$c_{\text{car.}} \cdot 10^{-3}$ [M/l]	Degree of β-carotene micro-crystalization	Windows preparation	Absorption anisotropy
1	2.0	5.0	high	rubbing	low
2	4.5	5.0	high	vacuum deposition	high
3	1.1	0.8	low	vacuum	high
4	1.1	0.8	low	isotropic plates	zero
5	4.5	5.0	high	isotropic plates	zero

the modulated light and the photoacoustic signal and as a result also the PAS amplitude q [38]. Measurements of the dependence of $q\nu$ against ν enable the evaluation of the decay time of the slow component of thermal deactivation [38,39].

3. Results and discussion

3.1. Absorption spectra and pigment orientation

Fig. 1a shows the absorption of the Chl a in ether and in the LC (set 4). The differences between the absorption spectra of the Chl a in ether and in the LC are related to the interaction of the LC with the pigment molecules. The change in the ratio of the Soret to the red-band absorption caused by embedding the Chl a in the LC cell was also observed previously [7]. The increase in the intensity of the vibronic bands at 509 nm and 540 nm is usually observed as a result of embedding the Chl a in the LC [8–10]. It is not caused by Chl a pheophytinization, because the red absorption band of the pheophytin is not observed. This conclusion is supported by MCD and absorption spectra analysis [9] which suggests that these bands are vibronic components enhanced by the interaction of the Chl molecules with the LC. The ratio of these bands to others can be used to gain information about the change in interaction of Chl a with LC. The red shift of the absorption maxima with respect to the maxima in ether are due to the LC solvent effect [8–10].

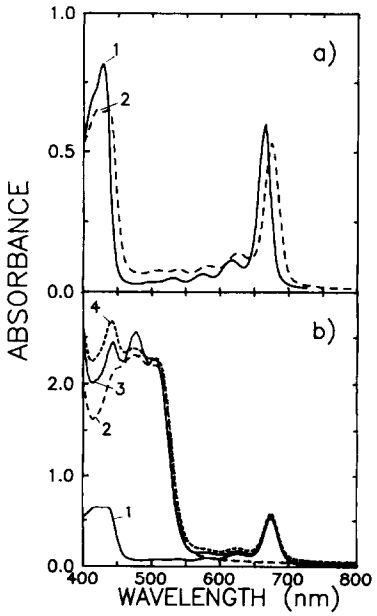

Fig. 1. Absorption spectra of investigated pigments (samples set 4). (a) 1 — Chl a in ether, 2 — Chl a in LC; (b) in LC, curves: 1 — Chl a, 2 — β-carotene, 3 — both pigments in the same cell, 4 — calculated sum of separated pigments absorptions.

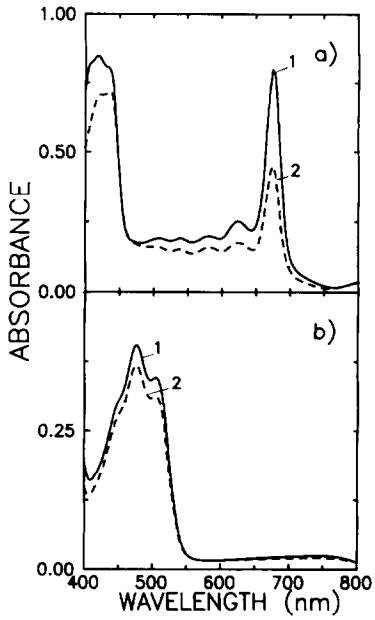

Fig. 2. Polarized absorption spectra (curves: 1 — parallel components, 2 — perpendicular). (a) Chl a (set 2); (b) β-carotene (set 3).

A comparison of the calculated sum of the absorption of Chl a and β-carotene located in separated cells (Fig. 1b, curve 4) and embedded in the same cell (curve 3) (recalculated with the same concentrations) shows that the absorptions of both pigments are not additive. The shape of absorption in a region of β-carotene in the presence of Chl a in a sample changes. It means that ground-state complexes between Chl a and β-carotene are formed. Fig. 2 shows polarized components of Chl a (Fig. 2a) and of the β-carotene (Fig. 2b) located between the same type of windows (set 3). Both pigments are oriented, but Chl a is in a higher degree. Linear dichroism ($LD = A_{\parallel} - A_{\perp}/A_{\parallel} + 2A_{\perp}$) of Chl a measured at

680 nm is LD = 0.23, whereas for β-carotene at 525 nm, LD = 0.04.

The cells prepared by windows rubbing (set 1) exhibit a much lower degree of orientation of the Chl molecules than the cells with orienting layers deposited in *vacuum* (Fig. 2b) [7–10]. The polarized absorption components taken at both polarization of light parallel and perpendicular to the cell axis are almost identical. This shows that there is not a strong axial orientation of the chlorine rings in the window plane, which means that the linear dichroism (LD) (0°, 90°) measured with respect to the cell axis at 0° and 90° vanishes. Only the sample containing both pigments exhibits some measurable LD (45°, 135°) which shows that the pigment axis of orientation is at an angle to the direction of the LC orientation axis (not shown).

For the cells with an SiO_x layer (sets 2 and 3) the LD is much higher than for set 1, but it diminishes strongly as a result of pigment mixing (not shown). It was unexpected that Chl *a* is oriented in a higher degree than elongated molecules such as β-carotene and that carotene can perturb the Chl *a* orientation.

Previously [23,24,31] it has been shown that in all model systems in which part of the molecules are in contact with water, three forms of Chl occur: 'dry monomer' (which are pigment molecules monodispersed and separated from the water molecules), 'hydrated dimers' (the affinity of Chl *a* to water is very high [29,30,39–41], therefore in order to avoid the occurrence of hydrated dimers special precautions have to be taken) and, at higher pigment concentrations, 'oligomers' built from hydrated dimers [29,31,40,41]. These forms differ in the position of their red absorption band. The chlorophylls in the lipid bilayer membrane can be treated as being almost dry [30], but the multilayers deposited on the CaF_2 substrate are partially hydrated [31]. The LC is really, a 'dry' solvent. Strong interaction between Chl and the LC prevents Chl aggregation [28,42,43]. Therefore even at the high concentration used (10^{-3} M) monomers predominantly occur (solvated by LC), with a low 'dry' dimers admixture. The position of the red band (671–675 nm), observed in both previous [8–10,27] and present samples, confirm this suggestion. The dependence of chlorophyll absorption on concentration was also investigated, but in the absorption spectra, changes which could confirm

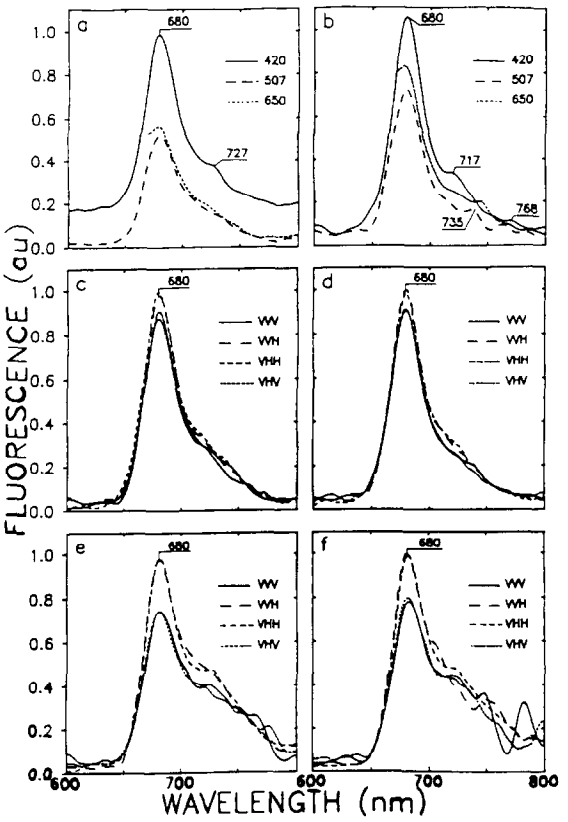

Fig. 3. Fluorescence spectra of pigments in LC (set 1). (a), (c), (e): Chl *a*; (b), (d), (f): Chl *a* with β-carotene. (a) and (b): dependence on wavelength of excitation (unpolarized light); (c)–(f): polarized spectra (notation explained in the text); (c) and (d): λ_{exc} = 420 nm; (e) and (f): λ_{exc} = 535 nm.

the formation of ground-state Chl aggregates were not measurable up to a concentration of 5×10^{-3} M. This could be related to the similar absorption spectra of the 'dry aggregates' and the monomers of Chl *a*.

3.2. Fluorescence and fluorescence excitation spectra

Fig. 3a,b shows the unpolarized fluorescence spectra of the samples of set 1 at various excitation wavelengths. Fig. 3c–f shows polarized fluorescence spectra of the same sample. Fig. 4 presents polarized fluorescence components of set 3. In the polarized fluorescence spectra the following notation is used: V = vertical, H = horizontal; the first and last letters

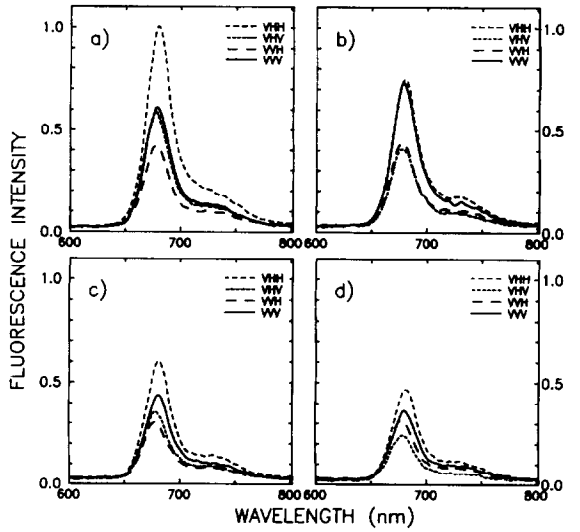

Fig. 4. Fluorescence spectra (set 3). (a) and (c): Chl *a*; (b) and (d): Chl *a* with β-carotene. λ_{exc}: (a) and (b) 420 nm; (c) and (d) 535 nm; (polarization notation given in the text).

describe the polarization of the excitation and emission beams, respectively. The letter in between denotes the direction of the sample axis. The samples of set 1 exhibit very low anisotropy of absorption, but, as follows from Fig. 3, emit polarized fluorescence. For these samples the ratio of the emission in the longer wavelength shoulder (at 727 nm) to that at the main maximum (at 680 nm) depends on the β-carotene addition at 507 and 535 nm excitation and on the polarization of the component. For unpolarized spectra the ratio of $F(727)$ to $F(680)$ is in a sample with Chl *a* alone equal to 0.28 and it diminishes to 0.18 as a result of β-carotene addition. The dependence of this ratio on polarization shows that interaction between Chl *a* and LC depends on pigment orientation with respect to LC oriented matrix. This ratio is low when the Chl is not aggregated [12]. The decrease in the shoulder to the main maximum ratio observed for the pigments mixture with an excitation at about 500 nm, where the absorption by both pigments is comparable, suggests the disaggregation of the Chl *a* as a result of the β-carotene excitation. The change in the polarization suggests that the new species are differently oriented than those present before the β-carotene addition.

The polarized fluorescence spectra of Chl without and with β-carotene addition, excited at 420 nm and 535 nm show that the β-carotene addition changes the polarization of the Chl *a* fluorescence more strongly at 535 nm than at 420 nm excitation. In the Soret band the contributions of the B_y and B_x transitions are strongly overlapped which means that the interpretation of polarized spectra excited in this region is complicated. It can be seen from Fig. 3e,f that for Chl alone the perpendicular polarized components (VVH and VHH) are higher than the parallel polarized components (VVV and VHV). The effect of the fluorescence polarization is due to photoselection by the polarized light because the pair of components obtained at different sample positions (VVV; VHV and VVH; VHH) are almost identical. This is in agreement with the low dichroism of absorption obtained for this set of sample. The negative polarization of emission decreases as a result of the β-carotene addition. The difference between the emission anisotropy of the Chl *a* alone and with the β-carotene shows that the addition of β-carotene generates differently oriented fluorescent species. Fig. 4 shows that the polarization of fluorescence of the type 2 sample is much higher than that of the type 1 sample. On the basis of Figs. 3 and 4, coefficients of emission anisotropy are calculated (Table 2). High negative values of r_d ($r_d = (VVH -$

Table 2
Anisotropy of fluorescence
$r_c = \frac{VVV - VHV}{VVV + 2VHV}$; $r_d = \frac{VVH - VHH}{VVH + 2VHH}$

Sample	λ_{exc} [nm]	r_c set 1 [a]	r_d set 1 [a]	r_c set 2 [a]	r_d set 2 [a]
Chl *a*	420	0.011	−0.005	0.013	−0.241
Chl *a* + β-car.	420	−0.005	0.007	0.208	−0.165
Chl *a*	535	0	0	0.077	−0.195
Chl *a* + β-car.	535	−0.009	0.005	0.150	−0.119

[a] For explanation of 1 and 2 see Table 1.

VHH)/(VVH + 2VHH)) obtained for set 2 show that the moment of absorption transition of exciting light is perpendicular to the transition moment of the observed emission and that the latter one is almost perpendicular to the LC orientation axis (VHH is higher than VVH). This again shows that strong B_x absorption components are located at 420 nm whereas at 680 nm a predominantly Q_y transition is emitted. The values of r_c ($r_c = (VVV - VHV)/(VVV + 2VHV)$) are positive but low, similar to those for samples type 1 having a low degree of orientation. In both cases the fraction of molecules with a low orientation of absorption TM with respect to the LC axis is responsible for r_c. The qualitative difference between r_c values of sample 1 and sample 2 is seen only at an excitation of 535 nm. The β-carotene addition causes a decrease in the negative value of r_d and an increase in the positive value of r_c. This shows the reorientation of Chl *a* molecules as a result of their interactions with β-carotene and the change in Chl *a* interaction with LC. Fig. 5a shows polarized fluorescence excitation spectra of the sample type 1, normalized in the main Soret peak. The β-carotene addition changes the polarized components ratio especially in the 535 nm band. The observed polarization of the excitation spectrum is predominantly due to polarized light photoselection. Fig. 5b shows polarized fluorescence excitation spectra of sample type 3. The β-carotene addition does not change the shape of the excitation spectrum observed at 680 nm and 720 nm (second not shown), but it dramatically diminishes the intensity of the observed fluorescence and causes a decrease in the fluorescence anisotropy of the oriented sample. It shows that carotene molecules form traps for the excitation of Chl *a*, competing with the fluorescence emission. In Fig. 5b is additionally shown the excitation spectrum of the same pigments mixture taken in natural light. In this case the $B_x(0,0)$ component located at the short wavelength side of the Soret band and the $Q_x(0,0)$ band at about 580 nm [7,8] are higher than at excitation with light polarized parallel to the LC orientation axis. It is because $Q_y(0,0)$ TM forms a lower angle with the LC axis than that of $Q_x(0,0)$ and $B_x(0,0)$ [7]. In the excitation spectra it is possible to recognize all the bands observed in absorption (Fig. 1). The fluorescence and the fluorescence excitation spectra suggest that in addition to

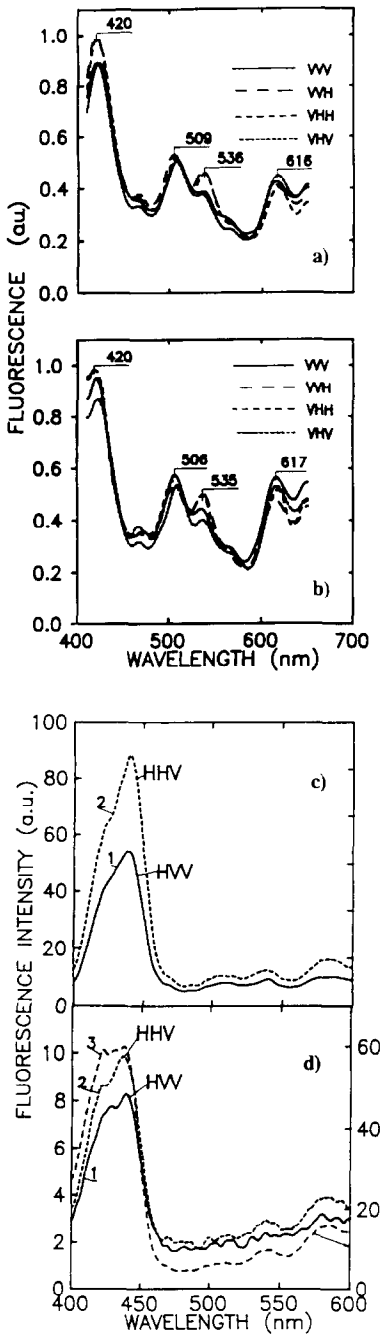

Fig. 5. Fluorescence excitation spectra. (a) and (b): set 1; (c) and (d): set 3; (a) and (c): Chl *a*; (b) and (d): Chl *a* with β-carotene; wavelength of observation 680 nm. In (d) curve 3 — in natural light (right-side scale). (Polarization given on the graphs).

the fluorescent Chl *a* forms some strongly deactivating excitation aggregates are also present, partially built with the participation of β-carotene. It is possible that the carotene and the Chl form specially oriented nonfluorescent or weakly fluorescent species or that the interactions between both pigments and the aggregates perturb the Chl *a* molecules orientation.

3.3. Lifetimes of fluorescence

Table 3 gives the lifetimes of the samples of set 1. The influence of the β-carotene addition depends on the wavelength of excitation and observation of fluorescence. With excitation in the red band of Chl *a* (at 670 nm) and observation of long wavelength emission ($\lambda > 780$ nm) β-carotene addition does not change the uniexponential decay of Chl *a* which has a lifetime (τ) of about 4470 ps. The τ obtained is strictly monoexponential with a value close to that of dry monomers. In the 670 nm region β-carotene molecules and Chl *a*–β-carotene complexes do not absorb. The concentration of Chl *a* in the measured samples with and without β-carotene was about 10^{-3} M. Previously [12] at a Chl *a* concentration (2×10^{-2} M) in the same LC matrix using excita-

Table 3
Fluorescence lifetimes of Chl *a* without and with β-carotene embedded in LC

Sample	Excitation λ_{exc} [nm]	Emission λ [nm]	τ_1 [ps]	τ_2 [ps]	χ^2
Chl *a*	380	680	4084 ± 8 (100%)		1.27
	380	680	4094 ± 8 (99.65%)	75.3 ± 24.0 (0.35%)	1.21
	380	> 715	4193 ± 10 (100%)		1.28
	532	680	3707 ± 26 (100%)		8.95
	532	680	3930 ± 14 (96.84%)	52.6 ± 5.5 (3.16%)	2.05
	532	> 780	3957 ± 12 (100%)		2.38
	532	> 780	4171 ± 16 (97.80%)	556 ± 44 (2.20%)	1.69
	670	> 780	4482 ± 29 (100%)		2.22
Chl *a* + β-car.	380	680	4104 ± 8 (100%)		1.58
	380	680	4382 ± 15 (93.67%)	1496 ± 146 (6.33%)	1.06
	380	> 715	4417 ± 12 (100%)		2.13
	380	> 715	4876 ± 32 (89.72%)	2055 ± 338 (10.28%)	1.61
	532	680	4714 ± 13 (100%)		1.46
	532	> 780	3992 ± 56 (100%)		2.47
	532	> 780	5126 ± 211 (95.18%)	859 ± 93 (4.82%)	1.34
	670	780	4462 ± 12 (100%)		3.11

tion in a Soret band, a similar value of mean lifetime of fluorescence equal to about 5 ns was obtained even at a strong emission in the 720 nm region suggesting pigment aggregation. This 4500–5000 ps component is due to Chl *a* molecules solvated by LC (monomers). The very low amplitude components necessary to improve fitting of the experimental decay (especially at 532 nm excitation) may be due to the small admixture of 'dry' Chl aggregates, ligands with traces of water or molecules differently connected with LC. On the basis of the presented data it is not possible to distinguish between these possibilities. The larger amplitudes of the short-time components at 532 nm excitation suggest that interaction with LC molecules plays an important role in the generation of this component. The aggregation of Chl in dry LC and in water soluble polymer (PVA) differs because the 'wet' Chl aggregates occurring in PVA are strongly fluorescent with a lifetime much shorter than that of monomers. A very low admixture of 'wet' Chl aggregates could be present even in the 'dry' solvent used in the present study.

The carotene addition changes the lifetime components. For the pigment mixture it is necessary to use a second component with a much higher amplitude and usually a longer lifetime than for a solution of Chl *a* only. It seems that from one side carotene prevents the Chl *a* aggregation, therefore the very short τ component is diminished, but also quenches the fluorescence of part of the Chl *a* molecules. Because emission of Chl *a*–β-carotene aggregates is not observed, these complexes can work as excitation traps. Because the pigment molecules in the anisotropic, viscous matrix are not randomly distributed, part of them can be energetically isolated from these complexes by mutual orientation and distances, whereas the second part may strongly interact with the 'traps'. The first part has a long lifetime (4100–5100 ps), the second, transferring excitation to 'traps', has a short fluorescence lifetime. Exact values of components depend on excitation wavelengths and observations of emission. Deconvolution on the exponents is a mathematical procedure which without additional information about fluorescent species is not univocal. At any rate, the comparison of lifetime analyses of samples without and with β-carotene addition shows measurable differences between these two sets of data.

3.4. Photoacoustic spectra

Fig. 6a shows the photoacoustic spectra of the Chl *a*, β-carotene and a pigment mixture for set 5. In Fig. 6b is shown the spectrum of the same pigment mixture at a higher frequency of light modulation and Chl *a* in LC at a higher pigment concentration (from set 4). Positions and ratios of the intensities of the PAS bands differ slightly from those of absorption which show that various forms are present with only slightly different yields of TD in the samples. It is possible that Chl *a* TD depends on the orientation with respect to the LC matrix. The pigment molecules are not oriented identically because of the distribution of their orientation angles around the direction of preferential orientation of the LC molecules [42]. The shape of the photoacoustic spectrum measured for the LC solution containing the pigment mixture is different from that of the sum of the photoacoustic spectra measured for the dye in the separated samples. The shapes of the PAS spectrum of pigments and the pigment mixture depend also on the fre-

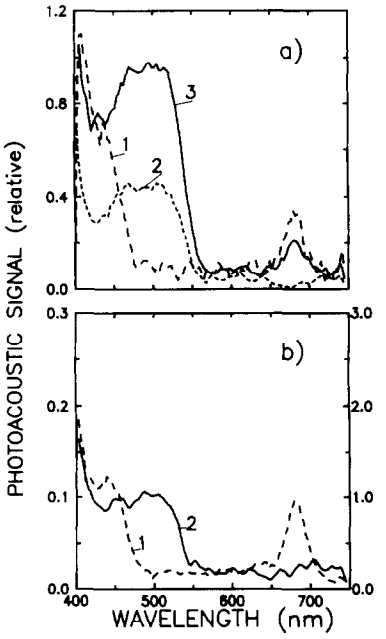

Fig. 6. Photoacoustic spectra. (a) Set 4, curves: 1 — Chl *a*; 2 — β-carotene; 3 — Chl *a* with β-carotene; frequency $\nu = 10$ Hz, phase shift $\phi = -177°$. (b) Curves: 1 — Chl *a* (set 5), $\nu = 10$ Hz, $\phi = -177°$ (right-side scale); 2 — Chl *a* with β-carotene (set 4), $\nu = 60$ Hz, $\phi = -177°$.

quency of light modulation (ν) which shows that the kinetics of thermal deactivation are different for the various pigments, and that these kinetics are changed as a result of the pigments introduction to the same sample. As an example of this effect PAS of Chl *a* with a β-carotene mixture (type 4) is shown in Fig. 6b. In this sample β-carotene is highly monodispersed. Because of the different yields of thermal deactivation of the Chl and the carotene and the different optical path lengths in the various samples, it is possible to obtain the values of TD only in arbitrary units and it is not possible to compare TD values for various sets of samples. The calculated data of TD for the main absorption maxima of Chl *a* and β-carotene for samples type 4 and 5 are shown in Table 4. From Table 4 it follows that for set 5 the TD (evaluated in the 436 nm and 675 nm ranges) for Chl *a* alone and that for Chl *a* with β-carotene are very similar. The much higher TD of carotene to that of Chl is in agreement with the lower yield of carotene fluorescence, than that of Chl *a*. The TD values for various carotene maxima are very different. In this set carotene was highly crystalized. For the pigment mixture TD in the carotene absorption region is still high, but values of TD of various maxima are changed. In the case of efficient singlet excitation transfer from carotene to Chl the value of TD in the carotenoids and the Chl *a* absorption region should be similar to that of Chl *a* alone. Therefore we can conclude that efficient ET from carotene to Chl *a* is not occurring. For set 4 in which both pigments are highly monodispersed Chl *a* located alone in LC exhibits a higher TD than in the presence of β-carotene. This may be due to the further diminishing in Chl aggregates concentration by carotene addition, because aggregates exhibit usually higher TD than monomers of Chl. Carotene alone exhibits in this set an unexpectedly low TD value but similarly as for the set 5 it is changed by Chl addition. Carotene is an 'LC-like' molecule and therefore should perturb the LC arrangement to a lower degree than Chl, which changes the arrangement in a large volume of its surroundings [44]. Microcrystals of carotene can perturb LC to a higher degree than monodispersed pigment. The different interactions of carotene and Chl with LC and various decay times of nonradiative deexcitation [38,39] could be the reason for the comparable TD of these two pigments located in mixed LC solutions and for the higher TD of Chl than that of carotene, when carotene is not crystalized (set 4). In a mixture of pigments the TD values increase slightly in the carotenoids region and decrease in the Chl absorption region. Because of the lower TD values the accuracy of the evaluation is lower for samples of set 4 than for samples of set 5.

The change observed in TD as a result of pigment mixing can be explained only by the formation of some aggregates of both pigments, exchanging excitation energy very efficiently into heat. The values are related not only to the competition between radiative and nonradiative deexcitation in separated molecules, but also to their interaction with the strongly anisotropic LC matrix and the different intensity ratio of slow to quick thermal deexcitation. The amplitude of PAS at a given frequency of light modulation and phase shift depends on the ratio of intensities of the slow (in μs or ms range) to the quick (ns or ps) decay components of TD intensities [35]. In order to check if the perturbation of Chl by

Table 4
Thermal deactivation (TD) yield [a.u.] (frequency of light modulation $\nu = 10$ Hz; phase shift $\phi = -177$)

Set of sample [a]	Pigments	Wavelength of TD calculation [nm]				
		436	443	472	502	675
4	Chl *a*	0.070	–	–	–	0.050
4	β-car.	–	0.020	0.020	0.020	–
4	Chl *a* + β-car.	0.029	0.029	0.035	0.041	0.030
5	Chl *a*	0.167	–	–	–	0.160
5	β-car.	–	0.620	0.240	0.180	–
5	Chl *a* + β-car.	0.170	0.180	0.550	0.320	0.130

[a] According Table 1 (TD accuracy ± 0.05).

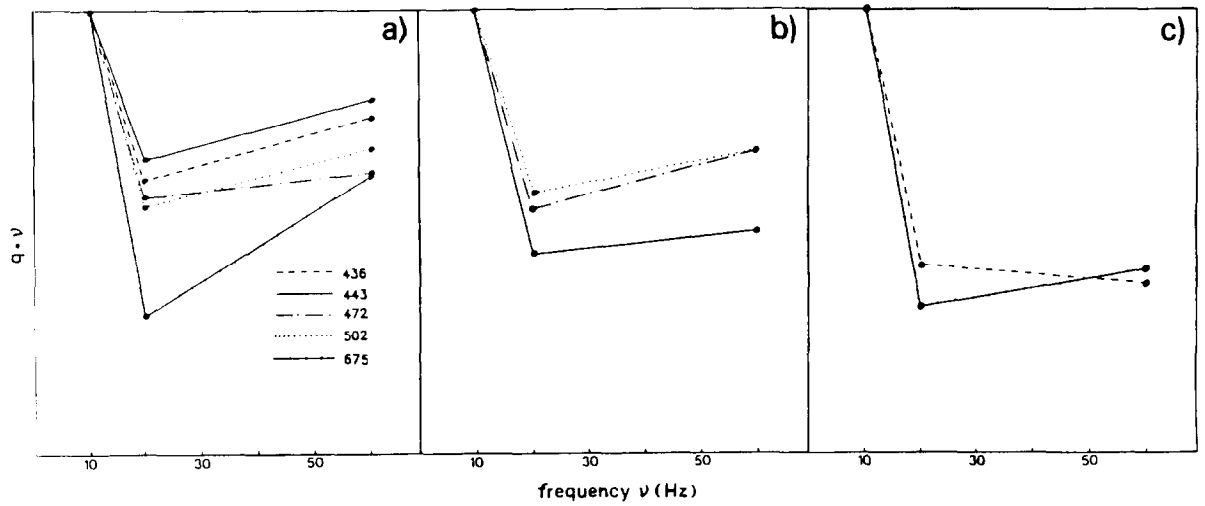

Fig. 7. Dependence $q\nu$ vs. ν (q — PAS amplitude; ν — frequency of light modulation). (a) Chl a with β-carotene; (b) β-carotene; (c) Chl a. Wavelengths given in Fig.

carotene can change this ratio, the dependence of $q\nu$ against ν is measured (Fig. 7). These graphs show that the participation of slow components of TD decay is changed as a result of carotene addition. This may be due to the change in the ratio of slow to quick intensities of the components or/and to the change in the slow component decay time [35]. The ratio of the red to the Soret PAS bands is the same at all used frequencies of light modulations, which shows that the signal is not saturated. Chl a triplet states a decay in the ms range, the carotene in μs [13]. The different slop of the mixture than that of separated pigments has to be due to the strong interaction of the pigments.

3.5. Delayed luminescence spectra

Fig. 8 shows the time resolved DL spectra of Chl a and Chl a with β-carotene in samples of type 2 and type 3. In both cases the Chl DL is quenched by β-carotene, but for sample 2 with β-carotene highly crystalized (Fig. 8a) and with a lower Chl a concentration (Fig. 8a) this quenching is stronger than that in sample 3 with a lower degree (type 3, Fig. 8b) of β-carotene crystalization. Also the kinetics of DL decay are different for samples with and without β-carotene, and different as a result of the addition of carotene in various degrees of crystalization (Fig. 9). The β-carotene alone and LC alone do not exhibit a DL signal outside the limits of accuracy. The excitation in the red band of Chl a shows the same kinetics of DL decay without and with β-carotene addition (Fig. 9). The strong influence of carotene on the DL intensity and the localization of

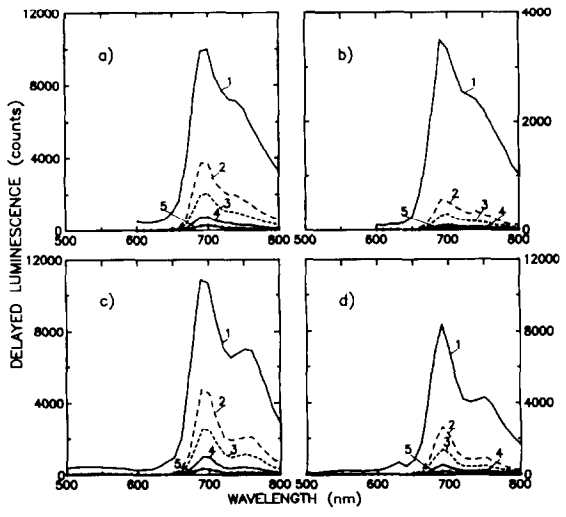

Fig. 8. Time-resolved delayed luminescence spectra. (a) and (c): Chl a; (b) and (d): Chl a with β-carotene. Wavelenth of excitation 420 nm. (a) and (b): set 2; (c) and (d): set 3. Time windows: curve 1 — 0.2–5.2 μs; 2 — 5.2–10.2 μs; 3 — 10.2–15.2 μs; 4 — 40.2–45.2 μs; 5 — 55.2–60.2 μs.

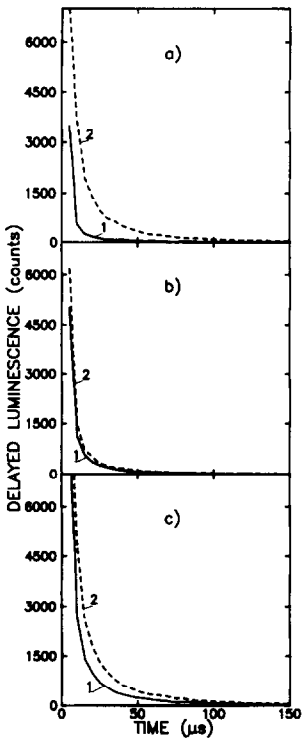

Fig. 9. Decays of DL measured at 680 nm. Curve 1 — Chl *a* with β-carotene; curve 2 — Chl *a* alone. (a): Set 2; (b) and (c): set 3. λ_{exc}: (a) and (c) 420 nm; (b) 650 nm.

the DL spectrum in the prompt fluorescence spectral region suggest that DL is a β-type delayed emission (with participation of triplet state and thermal activation from triplet to the excited singlet of Chl). This supposition will be checked by further investigations. The different influence of crystalized carotene in comparison to that of monodispersed is in agreement with TD data (Table 4).

4. Conclusions

(1) The addition of β-carotene to Chl *a* solution in LC changes several spectral properties of the pigment such as the lifetime of fluorescence, orientation in LC and the yield and kinetics of DL.

(2) The interactions between Chl *a* and β-carotene depend strongly on both the pigments' state of aggregation and their orientations.

(3) All samples with Chl *a* exhibit strong and slowly decaying delayed luminescence.

(4) The results can be explained by the supposition that nonfluorescent ground-state aggregates of both pigments are formed.

(5) The effective singlet excitation transfer from β-carotene to Chl *a* is not found even at very high pigment concentrations and high degrees of orientation. Pigments interact strongly between themselves, but the energy absorbed by Chl *a* in the presence of β-carotene is exchanged into heat instead of being transferred to the fluorescent form of Chl *a*.

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