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DICHROISM AND POLARIZED FLUORESCENCE OF CHLOROPHYLL *a*, CHLOROPHYLL *c* AND BACTERIOCHLOROPHYLL *a* DISSOLVED IN LIQUID CRYSTALS

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Three photosynthetic pigments were studied: chlorophyll *a*, chlorophyll *c* and bacteriochlorophyll *a* in nematic liquid crystal matrixes. The polarized absorption and fluorescence spectra as a function of the electric field have been measured. From the polarized components of the absorption $A_{\parallel}$ and $A_{\perp}$ of the pigments in liquid crystals two reduced components A_x and A_y are calculated (x and y are the direction of the axis which is going through the second, fourth pyrrol rings, and the first, third rings, respectively). From these results the orientation of chlorophylls in liquid crystals and the configuration of the transition moments in the skeleton of the pigment molecules were determined.

1. Introduction

The energy conversion in photosynthesis is initiated with light absorption by the photosynthetic pigments. The main light-absorbing pigments are chlorophylls: chlorophyll *a* (Chl *a*) in plants and algae and bacteriochlorophyll *a* (Bchl *a*) in photosynthetic bacteria. In addition, the living organisms contain various accessory pigments.

The photosynthetic apparatus is located in photosynthetic membranes with highly oriented chlorophylls. As follows for example from Förster's theory [1], the rate of the energy transfer depends on the mutual orientation of the transition moment direction of the donor and acceptor.

The anisotropic matrix is a very convenient model to study the influence of the oriented medium on the spectral properties of chlorophylls. The measurement of the chlorophyll properties in various anisotropic media became in the last years the interest of many authors [2–7].

Liquid crystal because of its fluid and oriented structure is also a good model system for studying the intrinsic anisotropic parameters of the pigment molecules. This system can give information on the transition moment configuration in the molecular frame.

In our previous paper, the influence of the LC matrix on the spectral properties of Chl *a* has been investigated [8–10]. In this paper the orientation of the transition moments with respect to the molecular skeleton of three pigments: Chl *a*, Chl *c* and Bchl *a* and also the interaction between these pigments and LC medium have been studied. For this purpose the polarization of the absorption band (linear dichroism), circular dichorism and the fluorescence spectra have been measured. The linear dichroism does not give definite information, if the absorption spectra contain overlapping bands or bands with vibronic structure. A good separation of the overlapping bands can be obtained with a reduction procedure proposed by Thulstrup, Michl and Eggers [11–14]. Previously this procedure was utilized for investigation of the polarized absorption of Bchl *a* in LC [15].

In this paper, based on the method of Thulstrup et al. the directions of the chlorophylls transition moments with respect to the orientation axis of LC were determined.

2. Materials and methods

Chl *a* and Bchl *a* were extracted from *Urtica dioica* and *Rhodospirillum rubrum*, respectively. Chl *c* was

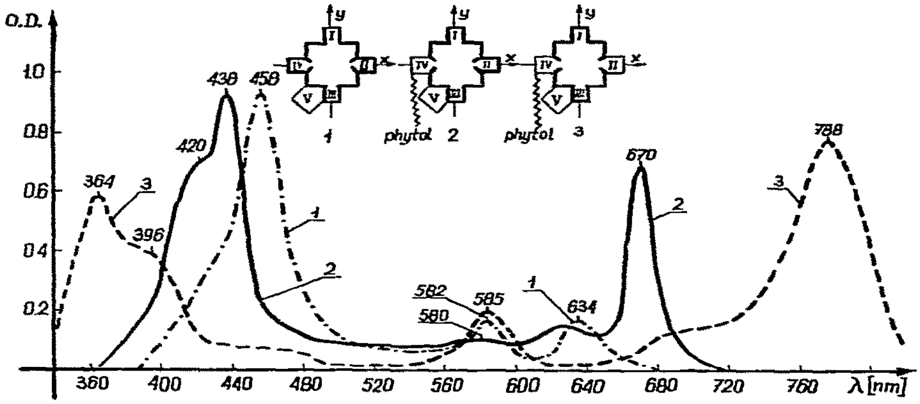

Fig. 1. Molecular structures of Chl *c* (1), Chl *a* (2), Bchl *a* (3) and absorption spectra of these pigments in LC: 1) Chl *c* in MBBA + EBBA, 2) Chl *a* in MBBA + EBBA, 3) Bchl *a* in PCB. (The thick lines in molecular structures represent the conjugated bonds system.)

obtained chromatographically from *Fucus furcatus* (the ratio Chl c_1/Chl c_2 is about 0.55 [16]).

The pigments were dissolved in three kinds of liquid crystal mixtures: p-methoxybenzylideno-p′-butylaniline (MBBA) + p-ethoxybenzylideno-p′-butylaniline (EBBA), p-pentyl-p′-cyanobiphenyl (PCB) and MBBA + EBBA + 4-dimethyl-amino-benzonitryl (DAB). These mixtures differ in the sign of the dielectric anisotropy: the first one is negative ($\Delta\epsilon < 0$) whereas the others are positive ($\Delta\epsilon > 0$).

Solutions were located in a specially constructed cell, as described previously [8]. In order to obtain an initial homogenous uniaxial orientation of LC and pigment molecules the Janning's method [17] has been used.

The absorption and the fluorescence spectra were measured as a function of the electric potential of 0–15 V applied to the cells.

Absorption spectra were carried out on a Cary 118 spectrophotometer equipped with polarizers. Fluorescence spectra were recorded with a device described previously [8] and CD spectra were obtained using a Dichrograph Mark III Jobin-Yvon spectropolarimeter.

The Thulstrup's method calculations have been carried out on a ODRA 1305 computer using a FORTRAN IV program.

The sample preparation causes a slightly different orientation of the matrix in the investigated cell with pigment compared to that of the reference sample. Therefore, the correction of the solvent effect was taken into account in the program applied.

3. Results

3.1. Absorption spectra

Fig. 1 shows the absorption spectra of Chl *a*, Chl *c* in MBBA + EBBA and Bchl *a* in PCB. The differences in the position and the intensities of the absorption bands of the investigated pigments arise from the differences in the molecular structure of the chlorophylls (the structure of Chl *a*, Chl *c* and Bchl *a* is shown in the upper part of fig. 1).

The symmetry of the conjugated bonds system is changing from D_{4h} (almost circular) in the case of Chl *c* to D_{2h} (rectangular) for Bchl *a*. Besides this, both Chl *a* and Bchl *a* have a phytol chain, which is missing in Chl *c*.

On the basis of the literature [18,19], one may suggest that for Chl *a* and Bchl *a* the *y*-direction of the absorption transition moment is going through the first and third pyrrol ring, whereas the *x*-direction is crossing the second and fourth ring.

Table 1
Linear dichroism of chlorophylls in LC

Pigment	Absorption band [nm]	$\frac{\mathrm{LD}}{A}$
Chl *a* in MBBA + EBBA	420	0.30
	438	0.17
	580	−0.12
	670	0.57
Chl *c* in MBBA + EBBA	458	0.20
	582	0.27
	634	0.10
Bchl *a* in MBBA + EBBA	585	−0.03
	788	0.34
Bchl *a* in PCB	364	0.13
	585	0.17
	788	0.13

3.1.1. Linear dichroism spectra

The polarized absorption components of Chl *a*, Chl *c* and Bchl *a* in MBBA + EBBA were measured. This measurement for Bchl *a* in the region of the Soret band is very difficult because of the high absorption intensity of the LC matrix and of the pigment arrangement perturbation. To circumvent this difficulty, Bchl *a* in PCB has been measured additionally. From the polarized absorption measurements one can calculate the reduced linear dichroism (LD) according to the formula [20]:

$$\frac{\mathrm{LD}}{A} = \frac{A_{\parallel} - A_{\perp}}{\frac{1}{2}(A_{\parallel} + A_{\perp})}, \tag{1}$$

where $A_{\parallel}$ and $A_{\perp}$ are absorption components when the electric vector of the light is parallel and perpendicular to the axis of the LC orientation, respectively, and A is the absorption as measured with unpolarized light on the same oriented sample to which the LD refers.

The results of the LD/A of chlorophylls in LC are listed in table 1. In the case of Chl *a* and Bchl *a* in MBBA + EBBA the largest value of the linear dichroism is obtained for the long-wavelength absorption band. It proves that the transition moment of these bands subtends the smallest angle with the orientation axis of LC.

Fig. 2 shows LD/A as a function of the applied potential. It follows that chlorophyll molecules are always oriented to some extent even if there is no electric field. The pigment and LC molecules interact with each other forming some kind of aggregate. In the electric field chlorophyll, being part of this aggregate, is reoriented together with the anisotropic matrix.

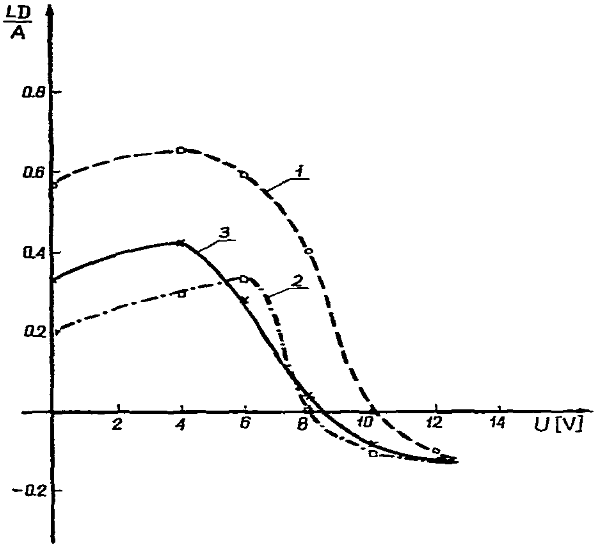

Fig. 2. Linear dichroism as a function of the voltage applied: 1) Chl *a* for λ = 670 nm, 2) Chl *c* for λ = 458 nm, 3) Bchl *a* for λ = 788 nm.

The initial preparation of the electrodes causes some angle of LC and pigment molecule axes with respect to the electrode surfaces. At low potentials the molecules, because of the LC negative dielectric anisotropy, tend to be oriented perpendicularly to the electric field, which is parallel to the cell surfaces. Therefore, $A_{\parallel}$ and also LD are increasing. The higher potential causes a perturbation of the orientation and as a consequence the LD is decreasing.

3.1.2. Separation of the overlapping absorption spectral bands

Separation of the overlapping absorption spectral bands of chlorophylls has been done according to the Thulstrup et al. approximation [11–14]. The following equations are used:

$$A'_y = A_{\parallel} - d_{\parallel}A_{\perp},$$
$$A'_x = A_{\perp} - d_{\perp}A_{\parallel}, \tag{2}$$

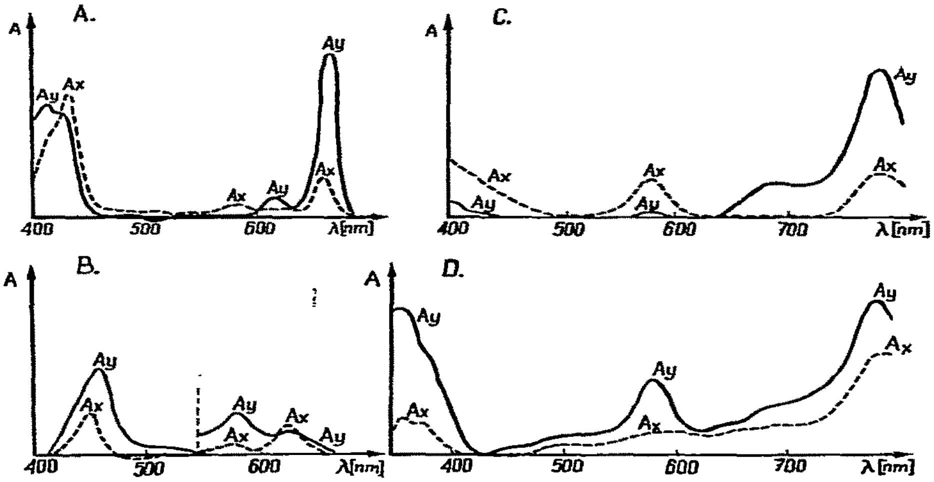

Fig. 3. Reduced components of absorption: A. Chl *a* in MBBA + EBBA, B. Chl *c* in MBBA + EBBA, C. Bchl *a* in MBBA + EBBA, D. Bchl *a* in PCB.

where A_y' and A_x' represent the total sum of light absorption due to "y-axis" and "x-axis" polarized transitions, respectively, $d_\parallel$ and $d_\perp$ are so-called "reduction factors" which are changing from 0 to 1 in the 0.1 step.

Fig. 3 shows the reduced absorption spectra of Chl *a*, Chl *c* and Bchl *a* in MBBA + EBBA and Bchl *a* in PCB obtained from equations:

$$A_y = A_\parallel - d_\parallel^0 A_\perp,$$

$$A_x = A_\perp - d_\perp^0 A_\parallel, \qquad (3)$$

where $d_\parallel^0$ and $d_\perp^0$ are values of $d_\parallel$ and $d_\perp$ which eliminate the contributions from $A_\perp$ and $A_\parallel$, respectively.

From fig. 3A it follows that the A_y and A_x components of Chl *a* are dominant in the short-wavelength and long-wavelength region of Soret band, respectively.

The band with maximum about 580 nm (fig. 1) has A_x character. In the long-wavelength absorption band (670 nm) both components A_x and A_y are overlapped, but the A_y component is stronger.

Similar data have been obtained in the case of Bchl *a* in the MBBA + EBBA (fig. 3C). The band with the maximum 585 nm has A_x character whereas the long-wavelength absorption band (780 nm) is a mixture of both the polarizations with the A_y component dominant.

The shape of the reduced absorption component of Bchl *a* in PCB is different from that in MBBA + EBBA (fig. 3D). The band with maximum at 585 nm has now a strong A_y character, but practically, the mixing of polarizations of the all absorption bands is observed. Similar results were obtained in the case of Chl *c*. Additionally, the measurement of the polarized absorption of Bchl *a* in the mixture of MBBA + DAB ($\Delta\epsilon > 0$) has been done. The reduced curves (results are not shown here) have a character close to the curves obtained for Bchl *a* in MBBA + EBBA ($\Delta\epsilon < 0$). These results give evidence for the independence of the shape of the reduced component of the dielectric anisotropy.

The reduction procedure allows to estimate the direction of the absorption transition moment with respect to the molecular frame. The angle between the direction of the given transition moment and the LC orientation axis is defined by the formula:

$$\mathrm{tg}^2\varphi_j = \frac{1/d_\perp^0 - b_j}{b_j - d_\parallel^0}\,\frac{2 + d_\parallel^0}{2 + d_\perp^0}, \qquad (4)$$

where b_j is the reduction factor for the individual peak j and is equal to $d_\parallel$ or $1/d_\perp$.

The values of the angles for the investigated pigments are listed in table 2.

Table 2
The angles between transition moments and axis of LC orientation for Chl *a*, Chl *c* and Bchl *a*

Pigment	Absorption band [nm]	The angle between transition moment and axis of LC orientation [deg]
Chl *a* in MBBA + EBBA	420	47
	438	62
	580	90
	670	0
Chl *c* in MBBA + EBBA	452	90
	462	33
	582	0
	634	46
Bchl *a* in MBBA + EBBA	585	90
	788	0
Bchl *a* in PCB	364	28
	396	90
	585	0
	788	38

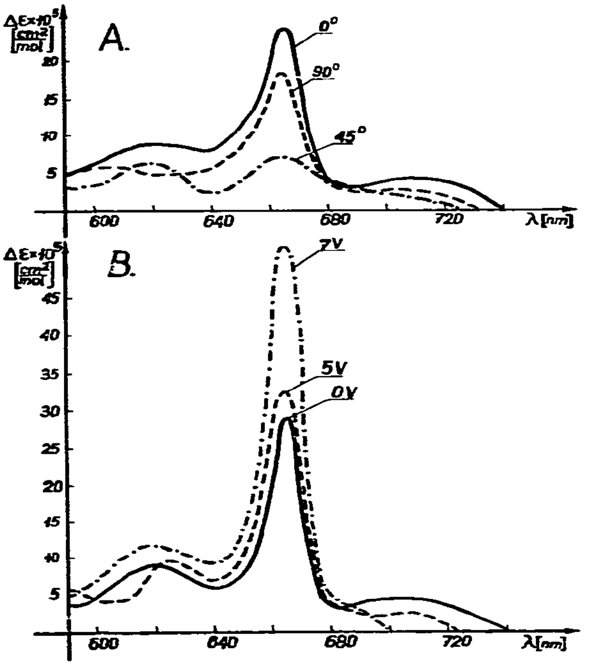

Fig. 4. CD spectra of Chl *a* in LC cell: A. for various angle, B. for various voltage applied.

3.1.3. Circular dichroism spectra

Circular dichroism (CD) is defined as:

$$\Delta\epsilon = \epsilon_L - \epsilon_R, \quad (5)$$

where ϵ_L and ϵ_R are the molar coefficients of the extinction of left and right circularly polarized light, respectively.

CD spectra of Chl *a* in MBBA + EBBA are shown in fig. 4. One can see that Chl *a* exhibits a high positive CD signal at 660 nm. The rotation of the cell around the axis parallel to the direction of the light beam causes changes of the magnitude of the CD signal (fig. 4A). The intensity of the CD signal is also changing with the applied potential (fig. 4B).

The CD measurement of the LC cell without pigment has been utilized for the determination of the arrangement of the molecules. The LC layer can be considered as a thin layer, in which the interference of light occurs. Fig. 5A shows the schema of the molecules arrangement for the cell without and with applied potential.

α_0 is the average tilt angle of the molecule axes in respect to the electrode surface. From the shift of the interference maxima or minima in CD spectra (fig. 5B), an initial tilt angle α_0 = 20° ± 2° was calculated.

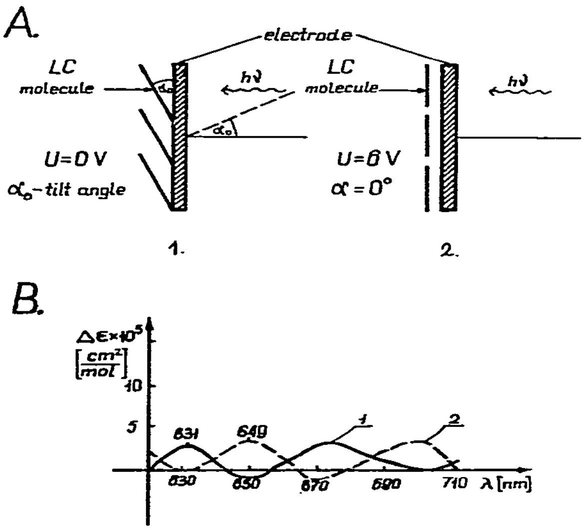

Fig. 5. Schema of the arrangement of LC molecules in cell (A) and interference in CD spectra for the coulorless LC (B): 1) without electric field, 2) with electric field.

3.2. Fluorescence spectra

The polarized fluorescence spectra ($F_{\parallel}$ and $F_{\perp}$) of Chl *a*, Chl *c* and Bchl *a* in the liquid crystals were measured.

From these measurements one can estimate the spontaneous polarization P and the degree of the orientation S [21]:

$$P = (F_{\parallel} - F_{\perp})/(F_{\parallel} + F_{\perp}) \qquad (6)$$

$$S = \frac{2P}{3(1-P)} \times 100\%. \qquad (7)$$

The obtained results are listed in table 3. The values of P and S are changing from sample to sample, therefore the results presented in the table 3 are averages.

Chl *c* in MBBA + EBBA has the lowest degree of orientation compared with Chl *a* and Bchl *a* in the same LC. The ordering of Bchl *a* in PCB is also very low.

Fig. 6 shows the polarized fluorescence components of Chl *c* for a potential of 0V and 6V (the electric vector of the excitating light beam is parallel to the orientation axis of LC). At such low potential (up to 6V) both parallel and perpendicular components increase. The changes of the fluorescence spectra in the electric field are smaller than that of the absorption.

The parallel and perpendicular fluorescence components of Bchl *a* in PCB and in MBBA + EBBA + DAB are shown in fig. 7. The positions of the fluorescence maxima of the components ($F_{\parallel}, F_{\perp}$) of Bchl *a* are shifted depending on the type of the LC. Applying the electric field, in the case of both solvent, the intensities of the parallel components are decreasing because both LC and pigment molecules are oriented perpendicularly to the electrode surfaces.

Table 3
Spontaneous polarization (P) and degree of orientation (S) of chlorophylls in LC

Pigment	P	S
Chl *a* in MBBA + EBBA	0.18	15%
Chl *c* in MBBA + EBBA	0.05	4%
Bchl *a* in MBBA + EBBA	0.14	11%
Bchl *a* in PCB	0.03	2%

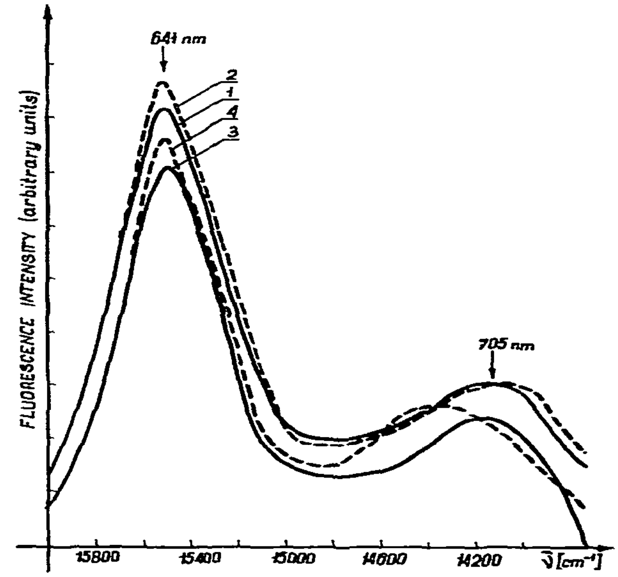

Fig. 6. Polarized components of the fluorescence of Chl *c* in MBBA + EBBA: 1) $F_{\parallel}$, 0V, 2) $F_{\parallel}$, 6V, 3) $F_{\perp}$, 0V, 4) $F_{\perp}$, 6V.

The observed fluorescence changes are different in PCB and in MBBA + EBBA + DAB. In the case of PCB, both parallel and perpendicular components decrease and for $U = 15$ V they reach the same value. For MBBA + EBBA + DAB only the parallel component is changing and even for 15 V it is slightly larger than the perpendicular one, which remains constant.

4. Discussion

The different orientations of the investigated chlorophylls which were observed without the electric field can be explained by:

1) various shapes of the conjugated bonds chain, and

2) different degrees of the molecular orientation.

The latter can come about by the different pigment –matrix interaction connected for example with the phytol chain.

Previously, many authors [18,19,22,23] basing themselves on theoretical considerations and experimental results have determined the polarization of

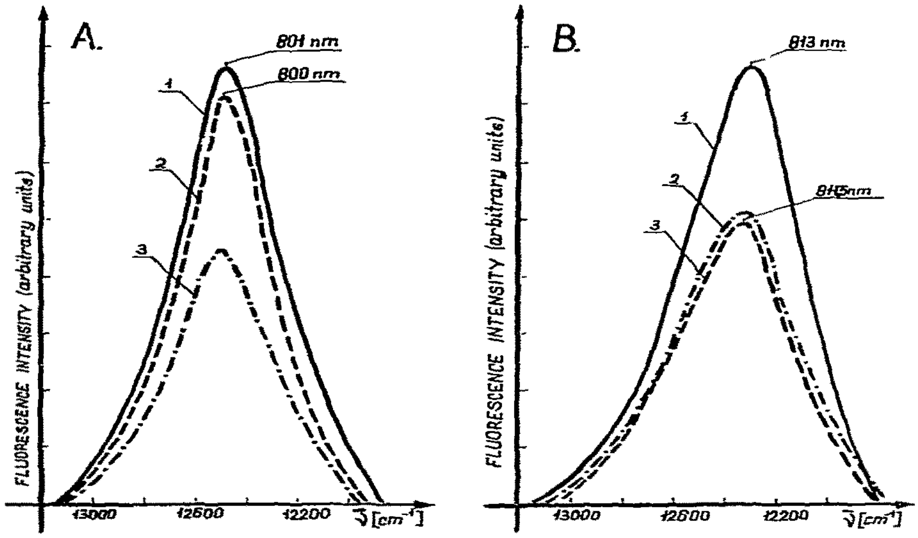

Fig. 7. Polarized components of the fluorescence of Bchl *a*: A. in PCB: 1) $F_{\parallel}$, 0V, 2) $F_{\perp}$, 0V, 3) $F_{\parallel} = F_{\perp}$, 15V. B. in MBBA + EBBA + DAB: 1) $F_{\parallel}$, 0V, 2) $F_{\parallel}$, 15V, 3) $F_{\perp}$ (unchanged with electric field).

chlorophyll absorption bands. Song [18] and Houssier and Sauer [19] have shown that the "red" absorption band of Chl *a* is clearly *y*-polarized; according to Seely [22] a small contribution of *x*-polarization is present in this band of the pigment. Our results, obtained with reduction procedure according to Thulstrup et al. [11–14] are in agreement with the suggestion done by Seely. The maximum at about 580 nm has *x* character, which is in good agreement with the literature [18,19,22,23]. The polarization of the Soret band of Chl *a* is assigned differently by various authors. According to Song's model [18], the main Soret band exhibits *y*-polarization, whereas its short-wavelength shoulder has *x*-polarization. According to Goedheer [23], the polarizations of the Soret band are reversed, whereas according to Houssier and Sauer [19] the main Soret band maximum is a mixture of *y* and *x* transitions. We have found that the short-wavelength region of the Chl *a* Soret band is predominantly *y*-polarized whereas the long-wavelength region is predominantly *x*-polarized.

Chl *a* in isotropic solvents has a small negative CD signal [19]. In the oriented liquid crystal cell this pigment shows a high positive CD signal similar to that observed for Chl *a in vivo* complexes and for chloroplast [24,25], but in our sample the magnitude of the CD signal is not connected with the structure of Chl *a* molecules. It is connected rather with the arrangement of the pigment molecules in the anisotropic medium, because the rotation of the cell around the axis parallel to the direction of the light beam changes the values of the CD signal. However, the CD signal is connected not only with the texture of LC but also with the perturbation by the electric field (fig. 4B).

Bchl *a* behaves differently in various types of LC. The orientation obtained both from LD and the spontaneous polarization of fluorescence measurements is much lower in PCB than that in MBBA + EBBA. The changes of the fluorescence intensity in the electric field for Bchl *a* in LC with positive dielectric anisotropy are also different by the use of different LC. For Bchl *a* in MBBA + EBBA + DAB the results obtained are similar to that for Chl *a* [10] and other pigments [26] in LC with $\Delta\epsilon > 0$. The change of the parallel as well as the perpendicular components of the fluorescence of Bchl *a* in PCB indicates a large angle between the average direction of the transition moment and the favourite axis of the matrix. The behaviour of Bchl *a* in MBBA + EBBA and in PCB is connected with the specific character of the interaction between pigment molecule and LC matrix. The mixture of MBBA + EBBA $[\mathrm{R}{-}\bigcirc{-}\mathrm{CH}{=}\mathrm{N}{-}\bigcirc{-}\mathrm{C_4H_9}$ $(\mathrm{R} = \mathrm{CH_3O}$ for MBBA, $\mathrm{CH_3CH_2O}$ for EBBA)] is very good in binding the hydrogen whereas in PCB $[\mathrm{C_5H_{11}}{-}\bigcirc{-}\bigcirc{-}\mathrm{C{\equiv}N}]$ the CN group will rather attach to the Mg atom (perpendicularly to the porphyrin ring). As a result of such arrangement of Bchl *a* molecules with respect to the PCB, the transition moment directions are changed with comparison to that in MBBA + EBBA even if no conformational changes are observed.

From the reduced curves of the absorption one can see that the band with maximum at 585 nm of Bchl *a* in MBBA + EBBA has *x* character what is also predicted theoretically [18,23]. The other character of the polarization is observed for this band with the change of LC (PCB): in this case the *y*-component is predominant.

According to literature suggestion [18,23], the long-wavelength band of Bchl *a* is polarized *y*-axis only. However, our results show that this band is a mixture of both *y*- and *x*-polarization. The contribution of the *x*-component in the case of Bchl *a* in PCB is much larger than in the case of Bchl *a* in MBBA + EBBA. Such various behaviour of Bchl *a* in different solvents gives evidence of the strong effect of the anisotropic matrix on the polarization properties of the pigment spectra. Similarly, a large perturbation by the matrix may occur *in vivo*: the anisotropic matrix may enhance the *x*-character of the red band substantially. For example, Pearlstein and Hemenger [27] assuming that the "red" transition in Bchl *a* *in vivo* is *x*-polarized rather than *y*-polarized have found good agreement with the experimental data. It is possible, that such polarization is connected with a specific interaction between the pigment molecule and the protein surroundings.

Chl *c* is the molecule with the highest symmetry of all investigated pigments and exhibits the lowest LD in the LC cell.

The changes of the absorption intensity in the electric field are larger than that of the fluorescence, which is in contrast to the results obtained for Chl *a* and Bchl *a*, previously [9].

The small value of the spontaneous polarization of Chl *c* obtained from the fluorescence measurements can be connected with either high symmetry of the emission oscillator or low orientation of this pigment in LC. The excitation with polarized light causes a small decrease of the polarization (fig. 6), however, the obtained value is lower than that in the case of Chl *a*. This indicates that the small value of polarization of Chl *c* is a result of both the high symmetry of the emission oscillator and the low orientation of this pigment in the LC matrix.

Until now no theoretical study concerning the polarization of the transitions of Chl *c* was undertaken. The high LD value for the band at 580 nm obtained from our results indicates that the direction of this transition moment subtends the lowest angle with the main axis of LC, whereas the angle between the direction of the red transition moment (634 nm) and LC axis is the largest.

However, from the reduced absorption curves one can see that all absorption bands are a mixture of both polarizations. These results suggest that there is no preference of any transition moment of Chl *c* in the plane of the porphyrin ring.

The angles estimated by the method of Thulstrup et al. only approximately reflect the real configuration of the transition moments in the molecule of Chl *c*. Because of the overlapping of the absorption band polarization in the case of this pigment as well as of Bchl *a* in PCB the accuracy of the Thulstrup's method is smaller than that for Chl *a* and Bchl *a* in MBBA + EBBA.

Comparing the results obtained for Chl *c* at one side, and for Chl *a* and Bchl *a* on the other side, we can draw the conclusion that in the pigment arrangement in the oriented matrix the phytol chain plays an important role. The value of the orientation degree of Bchl *a* and Chl *a* in MBBA + EBBA is similar one to another. However, in the case of Chl *c*, which is a molecule without phytol, the orientation is very low. It seems likely that in our model the phytol chain plays the same role in the pigment orientation as in the membrane model described by Brown and Wolken [28].

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