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INFLUENCE OF CHLOROPHYLL *a* ON INTERMOLECULAR INTERACTIONS IN LIQUID CRYSTAL

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Studies of the variability of the spectral properties (linear dichroism, polarized fluorescence and the energetic separation of the absorption and emission bands) of chlorophyll *a* in a nematic liquid crystal matrix with respect to the effects of solute concentration and the external electric fields were made. A close examination of the above mentioned types of variability suggest that the pigment molecules can influence a high initial order of the liquid crystal matrix. These structural changes of the matrix can be explained by the assumption that two types of chlorophyll with different orientations occur: the surface layers with the high degree of orientation, and a central volume of the sample with disordered molecules. The ordered molecules are sensitive to an reorientation by the external electric field.

1. Introduction

The essential roles of chlorophyll *a* (Chl *a*) in the primary process of photosynthesis are: the absorption of light, transfer of the excitation energy, and the charge separation for the subsequent electron transfer processes. Analysis of these processes requires an understanding of the structural organization of the chloroplast membranes. Anisotropic media simulating the chlorophyll orientation in a lipid matrix of the photosynthetic membrane may provide an information either about the transitions responsible for the molecular absorption and emission anisotropy, or about the various structural factors governing the orientation, (such as internal order, thermal motions, degree of pigment – solvent interaction, and so on).

A liquid crystal matrix (LC) appears to be an interesting model of a lamellar system because of its fluid and oriented structure. In such anisotropic media the pigment electronic energy levels are perturbated by the LC matrix state, mainly by the local electric fields produced by the dipole moments of nearby LC molecules. One can expect that matrix polarity should induce a large red shift of the absorption and emission bands, a broadening of the absorption bands, and a change in energetic separation of absorption and emission maximum. The influence of LC matrix and the local electric field on the spectral properties of pigment molecules has been demonstrated [1,2]. In this paper we have studied, on the bases of spectroscopic characteristics of Chl *a*, the influence of pigment molecules on the structural changes of the anisotropic matrix. We will show that both the perturbations in the degree of the orientation of LC molecules and the Chl *a* spectra are strongly pigment concentration dependent.

2. Material and methods

A solution of chromatographically purified chlorophyll *a* in liquid crystal mixture was located in a cell with windows of conducting transparent glass. A nematic liquid crystal with positive dielectric anisotropy (Field Effect Mixture, Eastman Kodak 11900) was used. Homogeneous alignment of the LC molecules was achieved by evaporation of an SiO_x thin layer onto the windows. The molecular alignment in such the LC cell was parallel to the substrate surface with liquid crystal director along the *z*-axis (fig. 1) and perpendicular to the substrate surface on applying a voltage. All the measurements were conducted at

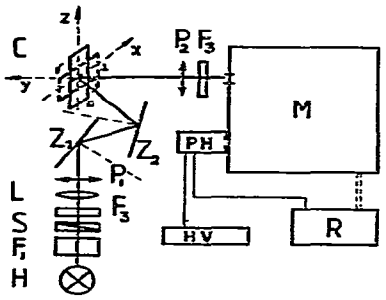

Fig. 1. Arrangement for measuring fluorescence spectra. C sample; H lamp; F_1 water filter; F_2, F_3 filters; P_1, P_2 polarizers; S shutter; L lense; Z_1, Z_2 mirrors; M double monochromator; Ph photomultiplier; HV power supply; R recorder.

room temperature, and an a.c. voltage of 1 kHz was applied. The sample thickness of 2.0×10^{-5} m was controlled by using a teflon spacer. The concentration of Chl *a* ranged from 3.4×10^{-4} to 4.0×10^{-3} M.

Details of the cell preparation and the absorption measurements were described elsewhere [1]. The experimental arrangement used for fluorescence spectra measurements is shown schematically in fig. 1. In such arrangement, excitation and fluorescence observation directions were both on the same side of the cell to diminish the reabsorption effects. The two fluorescence components ($F_\parallel$ and $F_\perp$) were measured by using vertically polarized of the excitation light in two positions of the cell (fig. 1). The fluorescence emission was excited at 436 nm with an interference filter (9 nm bandwidth).

Fluorescence lifetimes were measured with a phase shift fluorometer [3].

3. Results

The anisotropy nature of a liquid crystal–pigment system is determined by the orientation of pigment molecular structure (it depends on molecular "shape") and also by the degree to which the absorption transition moment of the dye is parallel to its molecular axis. Therefore, an observed spectral anisotropies of LC-pigment system depend on a geometrical shape of pigment molecule and its π-electron system symmetry [4]. In the case of Chl *a* (which has a porphyrin ring with a long phytol tail and π-electron system of D_{4h} symmetry [5,6]) some preferred directions of pigment molecules alignment were observed [1,2].

The molecular orientation is usually described in terms of an orientation tensor S [7,8], which reduces to a single S value if the pigment molecules have more than 2-fold symmetry axis (D_{nh}, $n \geqslant 3$) [9]:

$$S = \tfrac{1}{2}(3\cos^2\theta - 1), \qquad (1)$$

where θ is the angle between the long molecular axis and the LC director. In the case when the angle between the long molecular axis and the direction of the electronic absorption transition moment equals zero, the order parameter S is given by

$$S = (A_\parallel - A_\perp)/(A_\parallel + 2A_\perp), \qquad (2)$$

where $A_\parallel$ and $A_\perp$ denote parallel and perpendicular absorption, referred to the electric vector of light and the LC director. In our estimations of S the last equation and the two components of the Chl *a* absorption measured at 667 nm were used.

The change in the order parameter with the concentration of Chl *a* is shown in fig. 2. Results clearly demonstrate the decrease of order parameter with increasing the pigment concentration. Over the range of $10^{-4} - 10^{-3}$ M/l the order parameter decreases by 40% from its initial value. It indicates that increase of Chl *a* concentration introduces some kind of disorder in the LC matrix, changing a perturbation of Chl *a* molecules by the matrix.

The variations in absorption and emission spectra of Chl *a* can be explained in two ways: (1) as due to interaction between pigment and LC molecules or (2) as due to an increase in pigment–pigment interactions as a result of the increase of Chl *a* concentration.

The halfwidth of red absorption and fluorescence band of Chl *a* in the LC matrix against the pigment concentration are given in fig. 3. The halfwidth of the red absorption band (parallel component of absorption, $\delta_{A_\parallel}$) decreases with the increase of pigment concentration suggests that a great contribution to the absorption alterations arises from the interaction changes between pigment and LC molecules.

A similar conclusion may be drawn from fluorescence measurements. Fig. 4 illustrates the dependency of fluorescence intensity of Chl *a* on pigment concentration. The results from fig. 4 show almost linear rela-

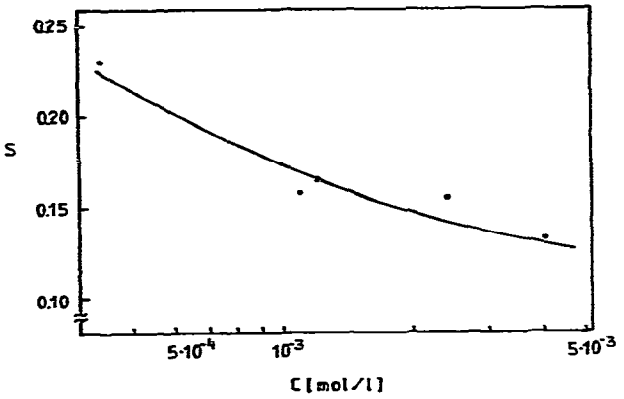

Fig. 2. Order parameter S of Chl a in the LC matrix versus concentration of the pigment.

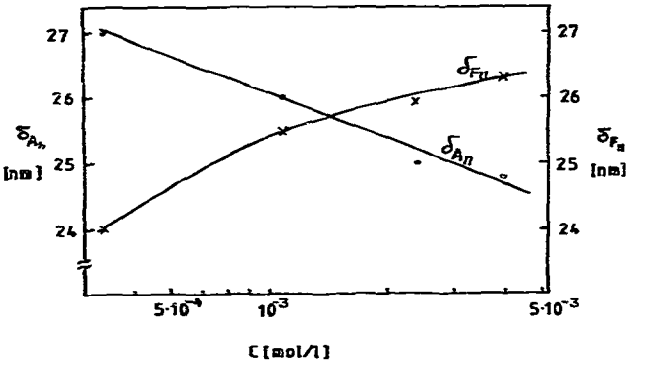

Fig. 3. Effect of the Chl a in the LC matrix on the half-width of the parallel components of absorption ($A_\parallel$) and of emission ($F_\parallel$).

tionship between the intensity of fluorescence polarized parallel to the LC director and the concentration of the Chl a. This means that a concentration quenching of Chl a fluorescence is over the investigated range of pigment concentration practically not significant. The above suggestion is confirmed by a dependence of the fluorescence lifetime on the Chl a concentration. At both positions of the cell (fig. 1) the lifetime of fluorescence was equal to 5.7 ± 0.2 ns, and was not dependent on pigment concentration.

The dependence of the energetic separation of the absorption and emission maxima, $(\nu_A - \nu_F)$, on pigment concentration is shown in fig. 5. A large increase of the energetic separation of the absorption and emission of Chl a is caused by a high concentration of the pigment. The halfwidth of fluorescence band is changed by increase of the Chl a concentration in a similar way (fig. 3).

With the aim of clarifying an average orientation of the absorbing molecules, the dependence of parallel and perpendicular components of the absorption and emission on the voltage applied, were measured. Since we have used the nematic liquid crystal with positive dielectric anisotropy, therefore with the external electric field the transition moments for absorption and emission should tip out of the polarization direction of the exciting beam and the polarization direction of the analysing polarizer, changing the absorption and emission intensities. The dependence of the two components of absorption and fluorescence

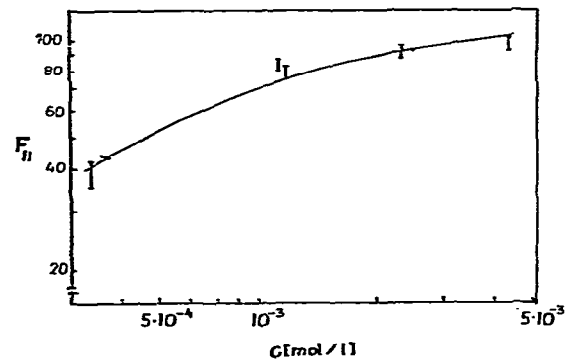

Fig. 4. Dependence of the fluorescence intensity (parallel component) on the Chl a concentration.

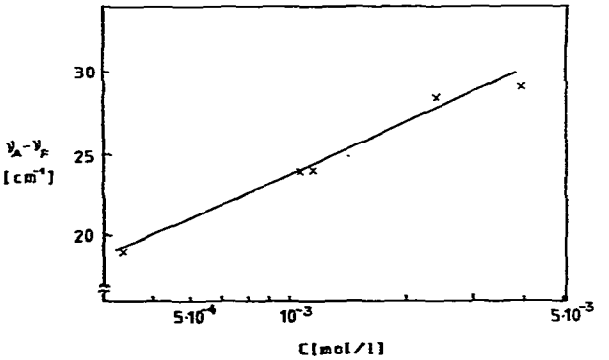

Fig. 5. Effect of the Chl a concentration on the separation of the absorption and emission maximum $(\nu_A - \nu_F)$.

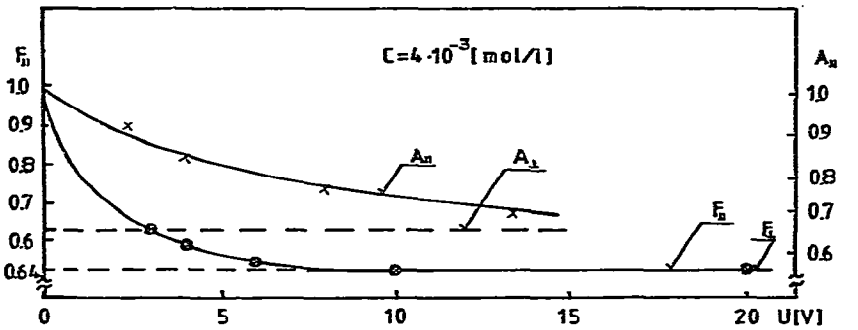

Fig. 6. Parallel and perpendicular components of absorption (A) and emission intensity (F) as a function of applied voltage.

on the voltage applied is shown in fig. 6. From these results it should be noted that the perpendicular components of both the absorption and emission are independent of the external electric field and, for an reoriented molecule by the external electric field, the parallel and perpendicular components have practically the same values. This means that: (1) molecules of Chl *a* are oriented initially in such a way that an average density of distribution of the transition moments responsible for the red absorption band in the cell volume is highest along the z axis (fig. 1). (2) The external electric field does not change the mean distribution of the transition moments along the x axis (fig. 6). (3) The average distribution of transition moments along the z axis reaches the x axis value after the molecular reorientation in the electric field.

The average distribution of the transition moments responsible for the red absorption band in the (x, z) plane (fig. 1) was investigated by measuring the absorption as function of the angle between the director of the cell and the polarization direction of the light traversing it. The results are shown in fig. 7.

4. Discussion

A simple model capable of explaining all our observations is shown in fig. 8. For the purpose of this model, we postulate the existence of surface layers (d_s) with a high degree of orientation and a central volume of the cell wherein, as a consequence of the thermal motions of the Chl *a* — LC molecule aggregates

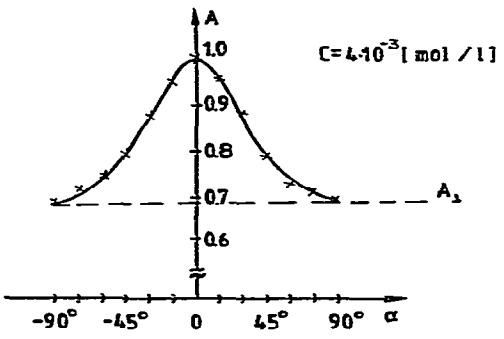

Fig. 7. Dependence of a normalised Chl *a* absorption on the angle between the director of the cell and the polarization direction of the absorbed light measured without the external electric field ($A_\parallel$ for $\alpha = 0$, $A_\perp$ for $\alpha = 90°$).

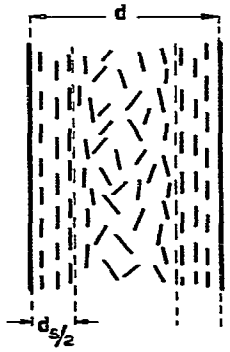

Fig. 8. A diagrammatic indication of the effect of Chl *a* concentration on organization of the LC molecules in the cell. d thickness of the cell, $d_s/2$ the thickness of the surface layer.

the nematic order of the matrix is damaged, and it practically represents some kind of disordered phase ("quasi-isotropic phase"). Because the increase of Chl *a* concentration causes a decrease of the order parameter (fig. 2) one can suggest that the thickness of the surface layer depends (if the cell thickness is kept constant) on the concentration of the Chl *a* molecules.

The model leads to predict the following effects, resulting from the perturbation of the highly ordered matrix by the increase in the Chl *a* concentration: (1) the mean dielectric anisotropy of the central volume of the sample should decrease to zero, and (2) the mean fluidity of the sample (described usually by its viscosity) should increase. Therefore the observed changes in the absorption and emission spectra reflect the variations in both above mentioned effects.

The broadening of the absorption band (described by $\delta_{A_{\parallel}}$ in fig. 3), beyond the amount due to thermal energy fluctuations, may be attributed to two phenomena [10]: (1) fluctuations in the interaction energy between the transition moment of the pigment and the electric fields produced by the dipole moments of nearby LC molecules, and (2) fluctuations in the interaction energy between the transition moment of the pigment and the electron polarization of nearby LC molecules. These both interactions give the strongest influence of solution on the pigment ($\delta_{A_{\parallel}}$ attains its maximum value) in the case of a perfectly anisotropic phase. Therefore with the decrease in the amount of the anisotropic phase one can expect that the half-width of the absorption band should decrease. The dependence of $\delta_{A_{\parallel}}$ on the Chl *a* concentration shown in fig. 3 confirms the above proposal.

The variations in the fluorescence spectrum depend on the interaction energy between the pigment molecules which are in their excited states and the dipole moments of nearby solvent molecules. Since that interaction energy depends on the ratio of the fluorescence lifetime and the mean time of dielectric relaxation, the measured variations in the fluorescence spectrum are mainly due to changes in the fluidity of the sample which affects the dielectric relaxation. According to dielectric Debye's theory a linear relationship exists between the mean time of the dielectric relaxation and the viscosity of the sample. In our case, the increase in Chl *a* concentration causes an increase in fluidity (decrease in viscosity) of the sample and this involves a decrease of the relaxation time. Since the lifetime of fluorescence was unaffected by a change in Chl *a* concentration, it seems highly likely that the increase in both measured values ($\delta_{F_{\parallel}}$ in fig. 3, and $\nu_A - \nu_F$) in fig. 5) is due to the increase of the ratio of fluorescence lifetime and mean time of dielectric relaxation.

According to the model, the anisotropic phase which is reoriented by the external electric field, exists mainly in the surface space (fig. 8).

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