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POLARIZATION OF BACTERIOCHLOROPHYLL ABSORPTION IN LIQUID CRYSTAL CELL

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To my dear Father and Professor Aleksander Jabłoński
for his 80th birthday

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From polarized components of absorption $A_{||}$ and $A_{\perp}$ of bacteriochlorophyll in nematic crystal solvent two reduced to molecular frame components A_x (directed through II and IV pyrrole rings) and A_y (through I–III rings) are calculated. Soret band splits into two components: shortwave A_y and longwave A_x. Band located at 580 nm has predominantly A_x character. The red band contributes light with both directions of polarization, but in various samples in different degree. Red band of bacteriochlorophyll is predicted to be polarized along y direction. Observed x-component can arise in a result of pigment perturbation by anisotropic medium. It is possible that similar effect can be responsible for low anisotropy of absorption of bacteriochlorophyll complexes in organisms.

1. Introduction

The Jabłoński method [1, 2] of studying dye molecules oriented in anisotropic matrices in now widely applied in biophysical investigations [3]. The aim of this paper is not only to explain the untypical behaviour in electric field of bacteriochlorophyll (BChl) in liquid crystal (LC) cell [4, 5], but also to attempt to explain the observation by other authors of low anisotropy of absorption of BChl [6] and chlorophyll a [7] in vivo.

In this purpose two reduced to molecular frame polarized components of absorption A_x and A_y are calculated from measured absorptions of light polarized perpendicularly ($A_{\perp}$) and parallelly ($A_{||}$) to the direction of LC orientation. It was predicted theoretically that BChl red band is done by Q_y transition and that it can be split into two components as a result of pigment dimerization [8], but without changing polarization. In agreement

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with literature [8], it is supposed that y-direction is going through I and III pyrrole rings, whereas x is crossing II and IV rings.

Q_x component of longwave absorption is usually located in 580 nm spectral region [8]. In oriented LC cell transition moment (TM) of chlorophyll main emission band is directed almost parallelly to the LC orientation axes [4]. Therefore it was an unexpected finding that red band absorption TM seems to be oriented differently, because of the lack of correlation between the relative changes in absorption $\Delta A/A$ and emission $\Delta F/F$ due to molecular reorientation in electric field [4, 5]. Dependencies $\Delta A/A$ and $\Delta F/F$ versus electric field intensity are varied with pigment concentration; its initial orientation degree of LC hydrogenation etc. [5]. Therefore, they are different in various cells, but in any cell, exact correlation between absorption and emission changes is not found. Differences between courses of absorption and emission changes are usually bigger in a case of BChl, than for chlorophyll a cells [5]. Similarly in isolated from bacteria complexes, the low BChl dichroism of absorption was found [6], which was explained by contributions from the groups of differently oriented pigment molecules, because it is known [9] that polarization of emission of similar preparations is higher.

Obviously in every molecule longwave absorption and emission transition moments are directed similarly to one another. But for sample containing fractions of pigment which are differently oriented or perturbed by surroundings and having also various yields of fluorescence, the average directions of TM obtained from absorption and emission measurements cannot be identical.

There are two tentative explanations of the lack of correlation between anisotropy of absorption and emission of BChl in model [4] and natural [6] systems: (1) Different fractions of pigment are differently oriented or, (2) Absorption and emission transitions are differently perturbed by surroundings.

It is known that perturbation of vibronic transitions by anisotropic medium influences predominantly the polarization of the spectrum [10]. Perturbation of excited states is usually stronger than that of ground states. In both (1) and (2) cases, in agreement with our previous observations [4, 5] the pigment concentration and its initial orientation have influence on the effects, but only in the second case, the shape of reduced polarized components could be affected.

2. *Measurements, results and discussion*

Chromatographically purified BChl was dissolved in nematic LC mixture (p-methoxybenzylidiene p-butylaniline (MBBA) + p-ethoxybenzylidiene p-butylaniline (EBBA)) and located in a cell with windows made of conducting glass. By special preparation of inner surfaces of windows, the LC and pigment molecules were oriented uniaxially even without electric field [4, 11]. Orientation axes are parallel to window surfaces. Details of cell preparation and the course of spectral measurements were done previously [4].

The absorption of light with electric vector parallel ($A_{\parallel}$) and perpendicular ($A_{\perp}$) to the LC orientation axes were measured for set of cells coloured by BChl in respect to similar cells containing only LC. The following three concentrations of BChl in LC were prepared: 2×10^{-3} M/1, 4×10^{-3} M/1 and 6×10^{-3} M/1. Values of the initial linear dichroism $\left(\mathrm{LD}=\dfrac{A_{\parallel}-A_{\perp}}{\frac{1}{2}(A_{\parallel}+A_{\perp})}\right)$ measured in maximum of red absorption band for investigated cells were 0.12; 0.64 and 0.34 respectively. LC orientations in coloured and reference cells are slightly different [5], which can have some influence on the shape of short wave length part of Soret band, because of LC absorption located in this region [5].

The procedure proposed by Thulstrup, Michl and Eggers [12, 13] were used to calculate A_x and A_y absorptions in molecular frame from experimentally obtained $A_{\parallel}$ and $A_{\perp}$. Calculated in this way, components A_x and A_y are proportional to the absorption in x and y directions in BChl ring, but the proportionality factors are not the same [12, 13]. Therefore, only shape of every component (Fig. 1) can be discussed, but not A_x/A_y intensities ratio.

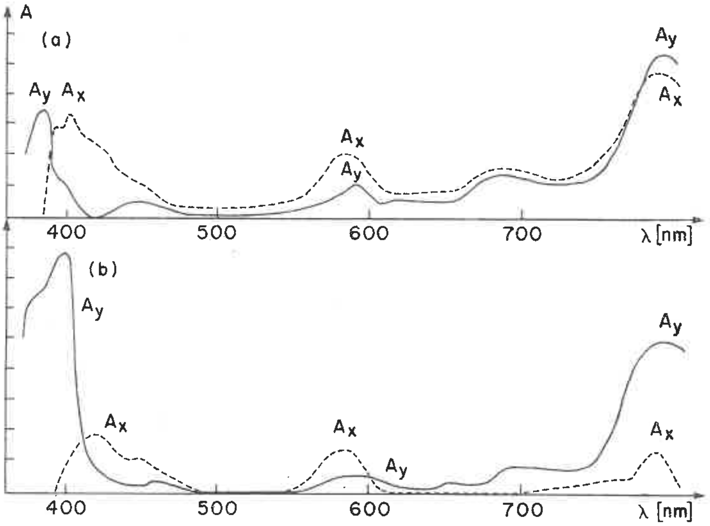

Fig. 1. Reduced polarized components of absorption of bacteriochlorophyll in liquid crystal. Concentrations: (a) 2×10^{-3}M/l, (b) 4×10^{-3}M/l. Components at 6×10^{-3}M/l similar to (b)

In shortwave region of Soret band practically only A_y appears. On the ground of literature [6] this absorption is supposed to have purely A_y polarization. Other spectral region polarizations were obtained by means of trial and error procedure [12, 13]. From orientation factors received in this way, it follows that BChl molecules are oriented uniaxially in LC cell, and that their behaviour is closer to predicted for rod-shaped molecules than to that of sheet-shaped. It is one more example, showing that it is not possible to predict the type of pigment orientation on the basis of its skeleton shape only, because "host-guest" interactions play an important role. As can be seen from Fig. 1 two polarized components in Soret band are well resolved having maxima at regions 380–390 nm and 400–410 nm respectively. In all cases, the band at 580 nm is in agreement with theoretical prediction and has predominantly A_x character.

In the region of red band, both A_x and A_y components contribute. A_x component intensity in red region is comparable with those in Soret band. In red region band A_x and A_y components are highly overlapped with one another, but in some cases (Fig. 1b) longwave region has stronger A_y character than shortwave edge of red band. This result can be considered only as the tendency of the shift between x and y components of red bands, but cannot be treated quantitatively. It seems that in a case of superposition of highly overlapping broad bands due to various pigment fractions, the shapes of reduced polarized absorption components give more information than formally calculated average angles between TMs in various spectral regions and the LC direction of orientation.

The course of components is changed from sample to sample (Fig. 1a and b) but always both polarized components appear in 750–800 nm region. This fact can be responsible for smaller changes in dichroism of absorption, than in anisotropy in emission as a result of molecular reorientation in electric field, because main fluorescence emission band is definitely polarized in y direction and only its small longwave shoulder has x polarization. Difference in properties of various cells can be only qualitatively explained as due to different contributions from components, because relative intensities of x to y components are not known. It is too early to speculate about pigment dimerization and even more so about the geometry of eventually formed aggregates on the ground of presented data.

Presented results suggest strongly that aggregation of BChl molecules with macromolecular surrounding can affect pigment anisotropy of absorption especially in a red band. This possibility can be taken into account in evaluation of pigment distribution in organisms on the ground of its LD measurements and a comparison of such results with those done for BChl solutions.

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