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Resonance Raman spectra of chlorophylls dissolved in liquid crystal matrices

III. Orientational distribution function of chlorophyll *b* in an MBBA + EBBA liquid crystalline matrix

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Polarized resonance Raman spectra of chlorophyll (Chl) *b* oriented in a mixture of *p*-methoxybenzylideno-*p*′-butylaniline (MBBA) and *p*-ethoxybenzylideno-*p*′-butylaniline (EBBA) have been measured. The spectra have been analyzed and the second- and fourth-rank order parameters and the orientational distribution function of Chl *b* are presented.

1. Introduction

It is now generally accepted that photosynthetic pigments bind to different proteins to form pigment-protein complexes which become embedded in lipid bilayers. The possibility cannot be excluded that lipids are also able to influence various processes through specific interaction with proteins, pigments and other lipids [1–3].

Although the study of intact chloroplasts and isolated pigment-protein complexes yields useful information concerning the orientation of pigments in biological membranes [4], one encounters difficulties connected with the problem of the overlapping signals originating from many chromophores in intact membranes. These difficulties are reflected by previously described data, for instance, reports on the orientation of chlorophyll (Chl) *a* and Chl *b* in black lipid membranes [5–8], which came to opposite conclusions. Also, the spatial arrangement of pigments is still obscure. Papers on the organization of Chl *a* and Chl *b* pigments in light-harvesting Chl *a*/Chl *b*-protein [9–12] do not provide compatible results.

It appears to be preferable to study a simpler ordered model simulating some aspects of the properties of a native system, such as a liquid crystal (LC) with incorporated Chl molecules, before investigation on an intact membrane can be performed. We do not claim that Chl is not attached to proteins in the membrane but we have used LC as an orienting matrix which allows us to change the orientation of the embedded pigments.

It is generally understood that Chl *b* plays an essential role in the processes occurring in green plants. Several optical properties and the orientational behaviour of Chl *a* and Chl *b* in model systems are very different [13–19]. In living systems they also interact differently with other molecules and differ with respect to aggregation properties as well [9]. In a previous paper [13], resonance Raman (RR) spectroscopy of Chl *a*

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showed that interpigment interactions are rather weak but the possibility cannot be excluded that the behaviour of Chl *b* is different. The orientation of Chl *a* in oriented LC systems has already been considered in many papers [14,15,17,18,20], however, the arrangement of Chl *b* is far less well understood. The aim of this paper is to explore the problem of the orientational behaviour of Chl *b* in an ordered system and it seems to be reasonable to compare the present results with those obtained previously for Chl *a* embedded in the same LC matrix [21].

The present study considers the ordering of Chl *b* molecules in a uniaxially oriented nematic MBBA + EBBA sample using polarized RR measurements and the orientational distribution function is constructed from second- and fourth-order parameters by use of information theory.

2. Materials and methods

Chl *b* was extracted from nettle leaves and column chromatographed on potato flour by utilizing the method described in ref. 22. The pigment was dissolved in an LC mixture: MBBA + EBBA (Riedel-de Haën), weight proportion 3 : 2. The sample was used without further purification. The concentration of Chl *b* in the LC was of the order of 10^{-9} M.

A solution of Chl *b* in MBBA + EBBA was placed in a glass cell of thickness 20 μm and macroscopic orientation of the LC was achieved as described elsewhere [23]. Preparation and measurements were carried out with the sample being kept under a stream of N_2 gas to avoid degradation of pigment and LC.

Raman measurements were performed at room temperature by means of a grazing excitation arrangement for two different experimental geometries as described in our previous article [21]. Resonance conditions for recording Raman spectra of Chl *b* in MBBA + EBBA were attained with an ILA-120 (Carl Zeiss Jena) argon-laser light of wavelength 476.5 nm with a resolution of 5 cm^{-1}. RR spectra were recorded using the Raman device described previously [24]. Polarized absorption spectra were measured with a Specord M40 spectrophotometer.

3. Results and discussion

Four polarized components of the RR spectra of the carbonyl region of Chl *b* embedded in the oriented MBBA + EBBA matrix are presented in fig. 1. This region of the spectrum involves the area spanning 1550–1700 cm^{-1}. There are two bands at approx. 1700 and 1661 cm^{-1} which appear to be associated with the vibration of the stretching modes of the free carbonyl ketone and aldehyde groups in Chl *b* molecules, respectively [13,25]. This allows one to investigate the orientational distribution of monomeric pigment in an oriented system. The bands located in the region between 1600 and 1550 cm^{-1} are due to the stretching motions in MBBA + EBBA [13,26–28]. The whole spectrum of Chl *b* in the same nematic LC for the bulk solution is presented and discussed in detail in the previous paper [13]. The

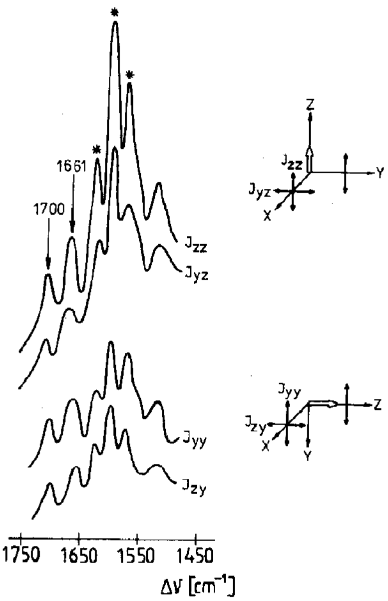

Fig. 1. Polarized RR spectra of the carbonyl region of Chl *b* in MBBA + EBBA. Left-hand part represents the experimental geometries for measuring (see ref. 5 for details). (*) Bands associated with MBBA + EBBA.

small difference, no greater than 1–2 cm^{-1}, between the maxima of the RR bands is within the experimental uncertainty.

In our previous paper on the order parameters of Chl *a* in MBBA + EBBA, we discussed in detail the reason for considering the vibrational Raman band at 1692 cm^{-1} as being associated with the stretching vibration of the Chl *a* ketone group [21]. For the same reasons, e.g., the absence of a contribution from LC scattering in the carbon region of RR spectra of Chl *b* and the 'purity' of the ketone and aldehyde group modes of Chl *b*, the bands at 1700 and 1661 cm^{-1} have been chosen for discussion. Because of band overlapping, Gaussian deconvolution was applied.

Some papers are available which deal with a theoretical approach to the order parameters evaluated on the basis of the Raman study [29–32]. In a previous paper, on the orientation of Chl *a* in an MBBA + EBBA cell, we have presented the assumptions and made the calculation of the second- and fourth-rank order parameters [21]. Here, we provide a summary of the method proposed in ref. 21. For a system with macroscopic uniaxial symmetry consisting of molecules of arbitrary shape, the Raman scattering tensor can be expressed by the elements related to the fixed molecular frame taking the formula evaluated by Jen [29]. It is well known that the Chl pigment is a planar molecule with the electronic transitions polarized in the plane of the macrocycle. Both electronic and vibrational transitions are usually associated with the limit molecular fragment and the transition moment directions are determined by the local symmetry of the molecule. If the vibration of interest is supposed to have uniaxial symmetry in the local frame and under the resonance condition used in our experiment, the Raman tensor of the vibrational mode can be reduced to having only one non-vanishing component according to ref. 33. The relation between the order parameters $\langle P_2 \rangle$ and $\langle P_4 \rangle$ and depolarization ratios can be used as shown previously [21]:

$$\langle P_2 \rangle = \frac{3r_2 + 3r_1r_2 - 4r_1}{3r_2 + 12r_1r_2 + 8r_1}, \tag{1}$$

$$\langle P_4 \rangle = \frac{3r_2 - 18r_1r_2 + 3r_1}{3r_2 + 12r_1r_2 + 8r_1}, \tag{2}$$

where r_1 and r_2 are depolarization ratios determined experimentally from the Raman scattered components, I_{YZ}, I_{ZZ} and I_{ZY}, I_{YY}, respectively (fig. 1). The subscripts of the terms in I refer to the polarization of scattered and incident light, respectively. The depolarization ratios r_1 and r_2 are corrected for Fresnel losses at the glass-sample boundary and self-polarization of the apparatus.

From eqs. 1 and 2, the $\langle P_2 \rangle$ and $\langle P_4 \rangle$ values are calculated. The 1700 and 1661 cm^{-1} bands of Chl *b* in an MBBA + EBBA matrix give $\langle P_2 \rangle = 0.165 \pm 0.015$ and 0.164 ± 0.015 and $\langle P_4 \rangle = -0.101 \pm 0.011$ and -0.065 ± 0.010, respectively.

It is clear that that linear dichroism experiment only yields the second-rank order parameter. The RR experiment gives not only the order parameter $\langle P_2 \rangle$, but also the order parameter $\langle P_4 \rangle$. The results show that while the order parameters $\langle P_2 \rangle$ are actually the same for the 1700 and 1661 cm^{-1} bands, the values of $\langle P_4 \rangle$ are different and negative in sign. The differences in $\langle P_4 \rangle$ for equal values of $\langle P_2 \rangle$ in two stretching modes of Chl *b* can be ascribed to differences in the location of two carbonyl groups in the LC matrix. However, we have obtained quantitatively similar results for Chl *a* in MBBA + EBBA with $\langle P_2 \rangle$ and $\langle P_4 \rangle$ being positive and negative, respectively [21].

The orientational distribution of the molecules in an oriented system can be characterized by the function $f(\beta)$ which is expressed in terms of even Legendre polynomials and is fully described when the order parameters are known. Nevertheless, from the Raman experiment, we obtain only the $\langle P_2 \rangle$ and $\langle P_4 \rangle$ order parameters which yield the truncated function $f(\beta)$ [21]. However, with the maximum entropy formalism [34,35], it is possible to obtain the broadest possible distribution function with the known values of $\langle P_2 \rangle$ and $\langle P_4 \rangle$:

$$f(\beta) = A \exp[\lambda_2 P_2 \cos(\beta) + \lambda_4 P_4 \cos(\beta)] \tag{3}$$

where A denotes a normalization constant and λ_2 and λ_4 are determined from the values of $\langle P_2 \rangle$ and $\langle P_4 \rangle$. The possible extremum of $f(\beta)$ is at an angle which can be calculated from $\cos^2\beta = -(1/2)\lambda\alpha$ where $\lambda = (3/2)\lambda_2 - (30/8)\lambda_4$ and $\alpha = (35/8)\lambda_4$ [36].

The distribution functions $f(\beta)$ for the Chl *b* stretching modes investigated are presented in fig.

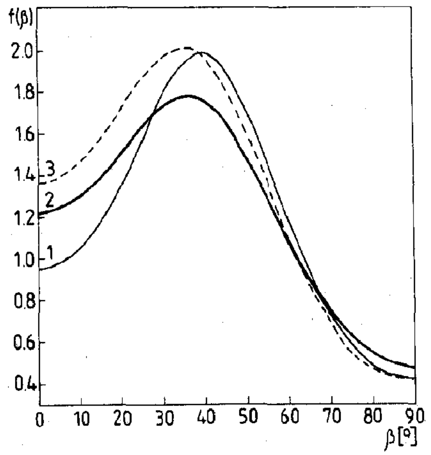

Fig. 2. The orientational distribution function $f(\beta)$ for: (1) the 1700 cm^{-1} band of Chl *b*, $\langle P_2\rangle = 0.165$, $\langle P_4\rangle = -0.100$; (2) the 1661 cm^{-1} band of Chl *b*, $\langle P_2\rangle = 0.164$, $\langle P_4\rangle = -0.065$; (3) the 1692 cm^{-1} band of Chl *a* (based on the data in ref. 5).

2. For the 1700 cm^{-1} band with $\langle P_2\rangle = 0.165$ and $\langle P_4\rangle = -0.100$ the information distribution function (curve 1) peaks at an angle $\beta = 40°$. The distribution function for the 1661 cm^{-1} band, on the other hand, with $\langle P_2\rangle = 0.164$ and $\langle P_4\rangle = -0.065$, exhibits a maximum at $\beta = 35°$ (curve 2). Curve 3 in fig. 2 has been constructed for the 1692 cm^{-1} band of Chl *a* in MBBA + EBBA on the basis of data in ref. 21. The β values obtained in fig. 2 correspond to $\beta = \arccos(-\lambda/2\alpha)^{1/2}$ which are equal to 39 and 36° for the 1700 and 1661 cm^{-1} bands, respectively. The corresponding value for the Chl *a* band at 1692 cm^{-1} is about 35°.

On the basis of fig. 2 we can compare the orientation distribution of two stretching modes of Chl *b* (curves 1 and 2) on the one hand and the distribution of Chl *a* and Chl *b* molecules on the other. Taking into consideration the curves in fig. 2, the similar but not identical character of the orientational distribution functions can be observed. All the functions have maxima lying between 30 and 40°. The position of the orientational distribution function maximum provides information on the probable direction of the transition moment of the stretching mode with respect to the orientational axis of the matrix. From fig. 2 it can be seen that the most likely direction for the 1700 cm^{-1} transition moment is 40° whereas for the 1661 cm^{-1} vibration mode preferential orientation is found at 35°. Moreover, it is known that the width of the distribution function is a measure of the mobility and degree of orientation of the molecules. The distribution functions presented in fig. 2 are broad, indicating the quite high mobility of the Chl molecules or their parts in an LC matrix. This result should not be unexpected when we take into consideration the shape of the Chl molecules and the interaction strength between them and LC molecules. On the one hand, the broad distribution function and the low orientational parameter can be connected with the low symmetry of Chl molecules and, on the other, with the weak interaction between Chl molecules and the LC matrix which is responsible for the orientation. The greater width of curve 2 compared to curve 1 in fig. 2 indicates that the mobility of the two carbonyl groups investigated in Chl *b* does not differ greatly.

A comparison can also be made for Chl *a* and Chl *b* in the studied LC. We note, taking into account the result in ref. 21 (curve 3), that $\langle P_2\rangle$ and $\langle P_4\rangle$ for corresponding bands (1700 cm^{-1} for Chl *b* and 1692 cm^{-1} for Chl *a*) are different. The perturbation of the spectra of Chl by peripheral substituents [37–40], solvent dielectric constant [19], coordination of the central Mg atom [41] and extent of aggregation [42] are well-known phenomena. Chl *b* differs from Chl *a* in the replacement of the methyl group in Chl *a* by an aldehyde group in Chl *b* at position 3 on ring II. It has been shown that the charge density of Chl *a* in its ground state is different from that of Chl *b* [43], which involves different dielectric properties [19] and thus different energy of interaction with solvent molecules, polarization of transition moments, etc. [15,44,45]. Moreover, the magnetic properties of Chls have been shown to differ [17,18,46]. It is believed on the basis of RR spectroscopy that the central Mg atom in the porphyrin ring of both Chl *a* and Chl *b* and the pair of free electrons in the LC molecule are responsible for the formation of some form of Chl-LC aggregate [13]. It was also shown that the 9C = O group in Chl *a* and the 9C = O and 3C = O groups in Chl *b* are essentially free in unoriented MBBA +

EBBA, e.g., they do not take part in the formation of aggregates with other Chl molecules [13].

From the present results, we can draw some conclusions on the orientational behaviour of Chl molecules in the LC investigated. Summarising both similarities and differences when comparing Chl *a* and Chl *b* and a number of their different features in the same LC [17–19,45,47], we can suggest that the different orientational behaviour in Chl *a* and Chl *b* in MBBA + EBBA is not only due to the interaction between the central Mg atom of Chl and LC molecules as has been observed in the bulk solution [13]. In spite of the peripheral groups being found not to participate in the formation of aggregates with other pigment molecules, some interactions between the aldehyde and/or ketone group of Chl and oriented LC molecules cannot be excluded. This interaction can be connected with the arrangement of Chl-LC which is forced by the orienting LC molecules, a fact which is reflected by the different orientation of Chl *a* and Chl *b* in the same LC.

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