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Resonance Raman spectra of chlorophylls dissolved in liquid crystal matrices

II. Order parameters of chlorophyll *a* in an MBBA + EBBA liquid crystalline mixture

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The molecular ordering of molecules embedded in a uniaxial liquid crystalline system has been considered. In particular, planar Chl *a* molecules in an MBBA + EBBA mixture have been studied by using polarized resonance Raman spectroscopy. The second- and fourth-rank order parameters and the orientational distribution function of Chl *a* in the nematic MBBA + EBBA liquid crystalline phase are discussed.

1. Introduction

Chlorophyll (Chl) *a* occurs as a major pigment in higher plants and many algae. Clearly, the elucidation of its spectroscopic features in vitro in both isotropic and anisotropic environments is of great importance.

In the first paper of this series [1], we studied the resonance Raman (RR) spectra of the bulk Chl *a* in an MBBA + EBBA sample which convinced us that Chl *a* in this liquid crystal (LC) exists largely as the solvated monomer even at the high concentration used (approx. 10^{-2} M). The magnesium atom is pentacoordinated with, presumably, an LC molecule as the fifth ligand. In this paper, we extend our previous data with the results obtained on the second- and fourth-rank order parameters of Chl *a* dissolved in oriented MBBA + EBBA by means of RR spectroscopy. The orientational distribution function of the molecules is then characterized by the order parameters $\langle P_2 \rangle$ and $\langle P_4 \rangle$.

In recent years, many studies have been published on the determination of the orientational order in different systems, for example, stretched polymer films, LCs and membranes [2–4], using several techniques based on polarized light spectroscopy [3] such as dichroism [5,6], fluorescence emission [7–9], ESR [10] and Raman scattering [11,12]. We have previously investigated the behaviour of chlorophylls in a number of different LCs [13–15].

The ordering of molecules in a macroscopically aligned uniaxial LC is one of the most popular methods of orientation. Such oriented systems can be utilized to estimate the second- and fourth-rank order parameters. Compared with absorption

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Abbreviations: EBBA, *p*-ethoxybenzylideno-*p'*-butylaniline; MBBA, *p*-methoxybenzylideno-*p'*-butylaniline.

spectroscopy and other conventional methods, the Raman technique provides simultaneously not only the second- but also the fourth-rank order parameters [12,16–18].

The theory of Raman depolarization in an oriented system has been discussed by a number of authors [19–22] but has been restricted to the case of molecules with cylindrical symmetry. It is interesting from a biological point of view to study the orientation of the porphyrin ring and we shall here consider a planar molecule.

We have evaluated the procedure for determining the second- and fourth-rank order parameters from measurements of the polarized RR spectra and shall discuss the orientation of Chl *a* molecules in a nematic MBBA + EBBA mixture. In a previous paper [23], we performed calculations, as did Journeaux and Viovy [24] of the second-rank order parameter for chlorophylls in nematic LCs using the fluorescence polarization method.

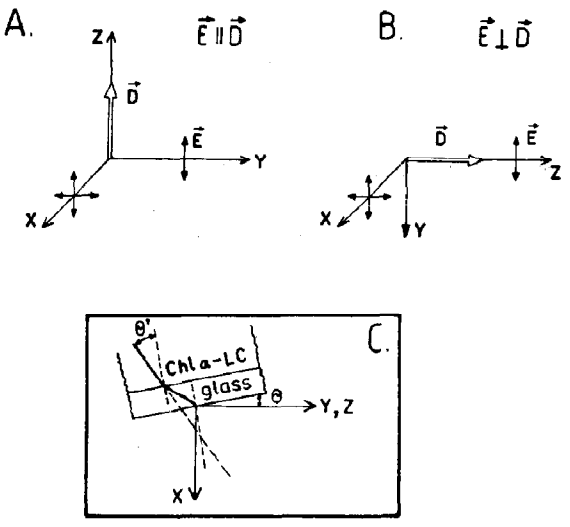

Fig. 1. Experimental geometries for RR measurements using the laboratory coordinate system *X*, *Y*, *Z*. (A) Parallel geometry with vertical $\vec{E}$ excitation parallel to the director $\vec{D}$; (B) perpendicular geometry with vertical $\vec{E}$ excitation perpendicular to the director $\vec{D}$; (C) path taken by the exciting light beam through the Chl *a*-LC sample.

2. Materials and methods

Chl *a* was extracted from spinach leaves and column-chromatographed on sugar powder according to the method of Omata and Murata [25]. The pigment was dissolved in an LC mixture: MBBA and EBBA (Riedel-de Häen), weight proportion 3 : 2. The sample was used without further purification. The concentration of chlorophyll in the MBBA + EBBA mixture was of the order of 10^{-2} M.

Macroscopic alignment of the LC was achieved by placing the solution of Chl *a* in MBBA + EBBA in a glass cell of thickness 20 μm as described elsewhere [26]. Preparation was carried out with the sample being kept under a stream of nitrogen gas in order to avoid degradation of Chl.

Raman measurements were carried out by means of the grazing excitation method ($\theta \approx 5°$) for two different experimental geometries as illustrated in fig. 1. The laser beam irradiated the sample along the *Y*- or *Z*-axis with the $\vec{E}$ vector parallel ($\vec{E} \parallel \vec{D}$) or perpendicular ($\vec{E} \perp \vec{D}$) to $\vec{D}$, respectively; the 'director' $\vec{D}$ refers to the axis of LC orientation in the sample. A half-wave plate was used to obtain a vertical position for the $\vec{E}$ vector. Scattered light was collected in the *X*-direction.

Resonance conditions for Raman spectra were achieved by employing light of wavelength 441.6 nm (He-Cd laser). The RR spectra were recorded at a resolution of 6 cm^{-1} at room temperature, using a double-grating Raman spectrophotometer and a multichannel analyser [27,28]. In order to reduce local heating of the sample at the portion being irradiated, the laser power was attenuated down to several milliwatts and focused onto the sample in the form of a strip. This experiment was performed under an atmosphere of nitrogen gas. Several recordings of the spectra were taken and no differences between recordings were found.

2.1. Method of calculation of the order parameters

Theoretical papers which make assumptions concerning the parameters of the molecular model have been published by Jen et al. [22] and Bréhamet [20]. The grazing method for measuring polarized RR spectra entails a more difficult calculation than that based on back-scattering

measurement [12]. Nevertheless, the grazing method is essential due to the requirements of high photosensitivity of chlorophyll pigments.

In our experiment the real angle of incidence into the LC sample is θ' ($0° < \theta' < 90°$) (fig. 1C). The general theory for polarized Raman experiments was developed by Bréhamet [20] for arbitrary incidence of the excitation beam on the sample. However, Bréhamet considered Raman depolarization with vertical and horizontal excitation. In our procedure, we employed only the vertical $\vec{E}$ component and therefore could not use Bréhamet's calculations.

In Raman experiments, the intensity of scattered light observed is proportional to

$$I = \left\langle \left| e^{(s)} \boldsymbol{R}_{lab} e^{(i)} \right|^2 \right\rangle, \tag{1}$$

where $e^{(s)}$ and $e^{(i)}$ are the unit vectors of polarization of scattered and incident light, respectively, $\boldsymbol{R}_{lab} = \{\alpha_{kl}\}$ ($k, l = X, Y, Z$) denotes the Raman scattering tensor (polarizability tensor derivative) based on the laboratory coordinate system X, Y, Z and the brackets represent an appropriate orientational average of the scattering molecules.

For the optical axis of the uniaxial LC to be aligned in the direction of the laboratory Z-axis (the Z-axis being parallel to the mean director) the scattered intensities observed in two experimental geometries (fig. 1) are as follows:
(1) parallel geometry ($\vec{E} \parallel \vec{D}$, fig. 1A):

$$I_{ZZ} = \left\langle \left| e_Z^{(s)} \boldsymbol{R}_{lab} e_Z^{(i)} \right|^2 \right\rangle = \left\langle \alpha_{ZZ}^2 \right\rangle, \tag{2}$$

$$I_{YZ} = \left\langle \left| e_Y^{(s)} \boldsymbol{R}_{lab} e_Z^{(i)} \right|^2 \right\rangle = \left\langle \alpha_{YZ}^2 \right\rangle, \tag{3}$$

for vertical excitation,
(2) perpendicular geometry ($\vec{E} \perp \vec{D}$, fig. 1B):

$$I_{YY} = \left\langle \left| e_Y^{(s)} \boldsymbol{R}_{lab} e_Y^{(i)} \right|^2 \right\rangle = \left\langle \alpha_{YY}^2 \right\rangle, \tag{4}$$

$$I_{ZY} = \left\langle \left| e_Z^{(s)} \boldsymbol{R}_{lab} e_Y^{(i)} \right|^2 \right\rangle = \left\langle \alpha_{ZY}^2 \right\rangle, \tag{5}$$

for vertical excitation.

Thus, for two common experimental sets we can obtain two independent depolarization ratios:

$$r_1 = \frac{I_{YZ}}{I_{ZZ}} = \frac{\langle \alpha_{YZ}^2 \rangle}{\langle \alpha_{ZZ}^2 \rangle}, \tag{6}$$

$$r_2 = \frac{I_{ZY}}{I_{YY}} = \frac{\langle \alpha_{ZY}^2 \rangle}{\langle \alpha_{YY}^2 \rangle}, \tag{7}$$

where the subscripts of the terms in I refer to the polarization of scattered and incident light, respectively.

As can be noted, under the experimental conditions we do not need to take into account the angle θ because there is no relationship between I_{ZZ}, I_{YZ}, etc., and the angle of incidence. Our results differ from those of Bréhamet [20]. In his paper it was shown that horizontal excitation made the calculation dependent on the angle θ. In our experiment, the arbitrarily small angle of incidence used gives rise to a decrease in excitation power ($I_V^{sample}/I_V^{air} = 0.13$). Therefore, for the above-mentioned reasons, we decided to apply the vertical $\vec{E}$ excitation and a direction of observation along the X-axis in order to avoid the calculations being dependent on the angle of incidence.

Under resonance conditions, the Raman tensor of the vibrational mode can be simplfied, leaving only a single non-zero component. Thus, the Raman polarizability tensor elements α_{ZZ}, α_{YZ}, α_{ZY} and α_{YY} in the laboratory frame can be expressed in the term for the molecular tensor element α'_{zz}, where z is the direction of the transition moment to the resonant electronic excited state.

With the above-mentioned assumptions we may use the formulae presented by Jen et al. [22] for α_{ZZ}, α_{YZ}, α_{YY} and α_{ZY}. Thus, from eqs. 6 and 7 we obtain:

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

$$\langle P_4 \rangle = \frac{3r_2 - 18r_1r_2 + 3r_1}{3r_2 + 12r_1r_2 + 8r_1}, \tag{9}$$

in which r_1 and r_2 are the experimental depolarization ratios. In the general case, the theory [20,22] involves not only the parameters $\langle P_2 \rangle$ and $\langle P_4 \rangle$ but also $\langle P_1 \rangle$ and $\langle P_3 \rangle$. However, taking into account the fact that the scattering tensor of the

Chl *a* mode is symmetric and has only one non-zero component, $\langle P_1 \rangle$ and $\langle P_3 \rangle$ disappear and the calculations become simpler.

Furthermore, correction for the boundary effect in media of different refractive indices due to Fresnel losses [29,30] and self-polarization of the apparatus must be borne in mind. The corrected depolarization ratios are as follows:

$$r_1^{\text{corr}} = \frac{(n_g + n_o)^2}{(n_g + n_e)^2} G r_1, \tag{10}$$

$$r_2^{\text{corr}} = \frac{(n_g + n_e)^2}{(n_g + n_o)^2} G r_2, \tag{11}$$

in which n_g is the refractive index of the glass plate and n_o and n_e represent the ordinary and extraordinary refractive indices of the LC sample, respectively. The G factor describes the depolarization arising from the apparatus.

3. Results and discussion

It is well known that the Soret and long-wavelength absorption bands of chlorophylls are due to in-plane transitions [31,32]. In a previous paper [23], we showed by means of linear dichroism that the Chl *a* transition moment associated with the Q_y band (670 nm) subtends the smallest angle with the molecular orientational axis. However, the RR spectra excited in the 'red' absorption region have never been presented because the lowest singly excited electronic state of Chl *a* is very radiative. Therefore, in this paper RR scattering was observed by using excitation close to the B_x (441.6 nm) Soret transition of Chl *a* in an MBBA + EBBA matrix. The RR experiment involving in-plane electronic transitions also makes the Raman active modes vibrate in-plane with respect to the conjugated bond system of chlorophylls.

The polarized RR spectra of Chl *a* embedded in an oriented MBBA + EBBA matrix are depicted in fig. 2. Four components correspond to two experimental geometries: A (curves 1 and 2) and B (curves 3 and 4) as shown in fig. 1.

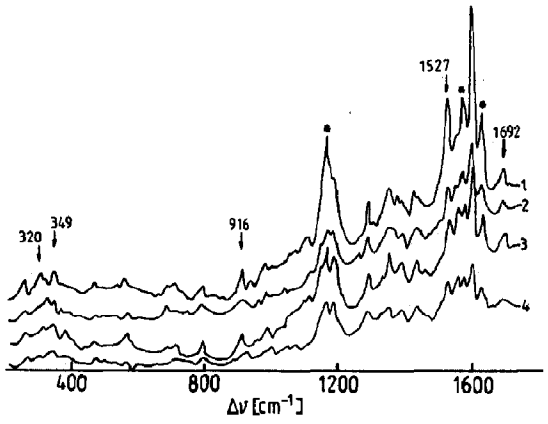

Fig. 2. Polarized RR spectra of Chl *a* in MBBA + EBBA: (1) I_{ZZ}, (2) I_{YZ} for parallel geometry, (3) I_{YY}, (4) I_{ZY} for perpendicular geometry; (*) bands associated with MBBA + EBBA.

The vibrational RR bands associated with the modes of the Chl *a* molecule are located between 50 and 1700 cm^{-1}. In the preceding paper [1], the RR spectrum, of a bulk Chl *a* MBBA + EBBA sample has been presented and discussed. In the present paper Chl *a* RR bands observed for ordered samples are slightly shifted with respect to those in the bulk solution [1,33,34]. These small differences, no greater than 2–3 cm^{-1}, are within the experimental uncertainty.

For further discussion we have selected bands taking into account the region in which MBBA + EBBA gives rise to no bands, or very weak bands if any, so the contribution of LC scattering can be neglected.

The depolarization ratios r_1^{corr} and r_2^{corr} defined in eqs. 10 and 11 are calculated by using the refractive indices $n_g = 1.525$, $n_o = 1.585$ and $n_e = 1.85$. From the calculated values of r_1^{corr} and r_2^{corr} we have estimated the two unknowns, $\langle P_2 \rangle$ and $\langle P_4 \rangle$, listed in table 1. In table 1, five bands at approx. 320, 349, 916, 1527 and 1692 cm^{-1} are listed. The 320 and 349 cm^{-1} bands, assigned to the modes involving in-plane motions of the magnesium atom in $Mg\text{-}N_4$ grouping [33,34], give $\langle P_2 \rangle = 0.280$ and 0.265 and $\langle P_4 \rangle = 0.160$ and 0.170, respectively. The 916 cm^{-1} band, associated with stretching vibrations of the C_a–C_b and CN bonds [33,34], results in values of $\langle P_2 \rangle = 0.290$ and $\langle P_4 \rangle = 0.026$. The strong band appearing at

Table 1

Calculated values of $\langle P_2 \rangle$ and $\langle P_4 \rangle$ for the five bands of Chl *a* in an MBBA + EBBA mixture

Band (cm^{-1})	$\langle P_2 \rangle$	$\langle P_4 \rangle$	Mode [33,34]
320	0.280±0.020	0.160±0.020	ν_{MgN}
349	0.265±0.020	0.170±0.020	ν_{MgN}
916	0.290±0.020	0.026±0.010	$\nu_{C_a-C_b}$, ν_{CN}
1527	0.320±0.030	0.085±0.010	$\nu_{C_a-C_b}$, $\nu_{C_a-C_a}$
1692	0.210±0.015	−0.070±0.010	9C=O

1527 cm^{-1} is assigned to C_b–C_b and C_a–C_b stretching modes in the phorbin ring [33,34]. We note that although the $\langle P_2 \rangle$ values for the 1527 and 916 cm^{-1} bands are in reasonable agreement with each other, those for $\langle P_4 \rangle$ are quite different. We believe that this discrepancy in $\langle P_4 \rangle$ can be ascribed to the fact that the 916 cm^{-1} band involves the C_a–C_b modes and is also convoluted with the CN stretching vibrations. On the other hand, the 1527 cm^{-1} band arises not only from the C_a–C_b vibrations but also from C_b–C_b vibrations which are indistinquishable in the RR spectra. The 1692 cm^{-1} band, attributed to the 9-keto carbonyl vibration, has also been taken into account, giving values of $\langle P_2 \rangle = 0.21$ and $\langle P_4 \rangle = -0.07$.

Analyzing table 1, one can say that in all cases the second-rank order parameter for Chl *a* in MBBA + EBBA is greater than 0.20 and more than one could expect. In particular, the parameter $\langle P_2 \rangle$ is found to be greater than the value we obtained in polarized fluorescence spectroscopy [23]. It is quite clear that the order parameters evaluated according to both methods should have the same values. We believe that this difference may be due to the fact that the laser beam probed a small region very close to the orienting glass plates where the local order is expected to be greater than the average for the sample [35].

Journeaux and Viovy [24] have also investigated the orientation of chlorophylls in MBBA and obtained a value of $\langle P_2 \rangle$ equal to 0.56 at 25 °C which is far greater than that which we obtained in ref. 23 and in the present article. The $\langle P_2 \rangle$ value in ref. 24 is rather close to the second-rank order parameter for pure MBBA, namely, 0.5–0.6 as shown by a number of authors [12,36]. We feel that the hypothesis of Journeaux and Viovy that chlorophyll pigments in an LC and pure LC have the same order parameter does not appear to be completely correct. This assumption is only applicable when the shapes of the LC and pigment molecules are similar, as has been demonstrated by Nakajima et al. [18] for β-carotene.

The distribution of molecules in uniaxially oriented systems such as the LC used here is characterized by the angle β made between the axis of molecular symmetry and the major axis of the sample using the distribution function $f(\beta)$:

$$f(\beta) = \sum_{L=0}^{\infty} \frac{2L+1}{2} \langle P_L(\cos\beta) \rangle P_L(\cos\beta); \quad (L\text{: even}). \tag{12}$$

This function is fully described when all parameters $\langle P_L \rangle$ are known. So far only second- and fourth-rank order parameters are available from experiments. Hence, $f(\beta)$ with $\langle P_2 \rangle$ and $\langle P_4 \rangle$ is truncated [4,37] but, nevertheless, provides us with a reasonable picture of the statistical distribution.

The vibrational Raman band at 1692 cm^{-1} associated with the stretching vibration of the 9-keto carbonyl group is the only band which is not disturbed by other vibrations. In consideration of the 'purity' of the keto group mode, the band at 1692 cm^{-1} has been selected for further discussion. The shape of $f(\beta)$ for this stretching mode is depicted, in fig. 3. Fig. 3 comprises two curves, curve 1 being plotted for the case when $f(\beta)$ is calculated by using the two non-vanishing elements, $\langle P_0 \rangle$ and $\langle P_2 \rangle$, of the power expansion in eq. 12, whereas curve 2 represents $f(\beta)$ reconstructed with $\langle P_0 \rangle$, $\langle P_2 \rangle$ and $\langle P_4 \rangle$ of eq. 12. From fig. 3 one can observe that if only $\langle P_2 \rangle$ is known the distribution has a maximum at $\beta = 0$ and decreases monotonically to a minimum at $\beta = \pi/2$. Curve 2 illustrates the influence of the fourth-rank order orientational parameter on that of the second rank.

For $\langle P_2 \rangle = 0.21$ and $\langle P_4 \rangle = -0.07$ obtained for the 1692 cm^{-1} band of the Chl *a* RR spectrum, the distribution function peaks sharply at an angle near 30°. This means that the collective molecular tilt is monitored by a maximum of the

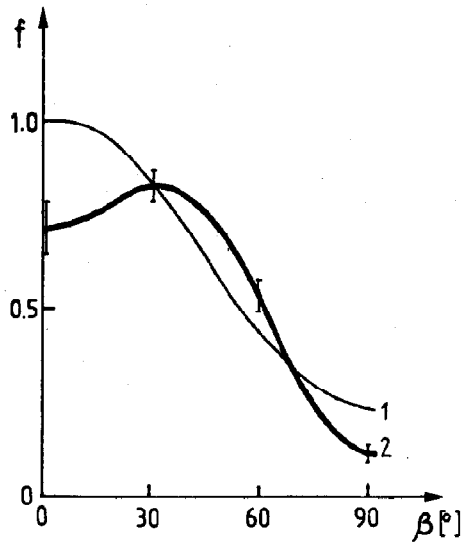

Fig. 3. Distribution function $f(\beta)$ for the 1692 cm^{-1} band of Chl *a*: (1) Calculated for the first two elements, (2) calculated for the first three elements (see text); $\langle P_2 \rangle = 0.21$, $\langle P_4 \rangle = -0.07$.

distribution at an angle intermediate between 0 and $\pi/2$. As is evident from curve 1 in fig. 3, when one takes into account the $\langle P_2 \rangle$ parameter only, most of the Chl *a* molecules in an MBBA + EBBA matrix are found at $\beta = 0°$. However, on including the parameter $\langle P_4 \rangle$, the maximum of the distribution function shifts to a β value of about 30° (curve 2). Knowledge of both parameters provides a more appropriate estimation of the distribution function for Chl *a* molecules in the LC matrix used. The β value of about 30° seems to be in good agreement with that obtained in our previous papers [23,38].

The question concerning the orientation of Chl *a* molecules MBBA + EBBA cannot be answered using this procedure, and therefore remains to be elucidated in the future.

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