Biophysical Chemistry 26 (1987) 91–99
Elsevier

BPC 01128

Resonance Raman spectra of chlorophylls dissolved in liquid crystal matrices

I. The interaction between chlorophylls and a liquid crystalline MBBA + EBBA mixture

Danuta Wróbel

Institute of Physics, Poznań Technical University, Piotrowo 3, 60-965 Poznań, Poland

Received 5 February 1986
Accepted 4 November 1986

Chlorophyll *a*; Chlorophyll *b*; Pheophytin *a*; Liquid crystal; Resonance Raman spectrum

The resonance Raman (RR) spectra of chlorophyll (Chl) *a*, Chl *b* and pheophytin *a* dissolved in a liquid-crystalline MBBA + EBBA mixture were measured. The RR frequencies of chlorophylls in the liquid crystal (LC) are compared with those of solutions of various chlorophyll monomers and aggregates taken from the literature. It is concluded that Chl *a* and Chl *b* in LC exist largely as the solvated monomers, even at high concentration. The magnesium atoms in both Chl *a* and Chl *b* are pentacoordinated.

1. Introduction

Chlorophyll molecules exist in photosynthetic organisms as the light-harvesting pigments and in the reaction centers as well.

In recent years studying the properties of chlorophylls in oriented systems by using different techniques for orientation has became the interest of many authors [1,2]. In this paper a nematic liquid crystal (LC) has been used as a matrix to obtain further information on the spectral features of chlorophyll (Chl) *a* and Chl *b* in such anisotropic surroundings.

We have previously studied the behaviour of chlorophylls in a number of different LCs [3–9]. The perturbation of the polarization properties of chlorophyll in this anisotropic environment was indicated [5,6]. However, the mechanism of interaction between chlorophylls and LC molecules has not yet been definitively established.

A particularly useful method to elucidate intermolecular interaction and also molecular structures and conformations in both in vivo and in vitro investigations is provided by resonance Raman (RR) spectroscopy [10–14]. The frequencies and intensities of RR bands make it possible to draw certain conclusions about the aggregation states of various chlorophylls. This technique detects enhanced vibrational spectra with high selectivity. It has been shown that the resonating modes include stretching motions of the 9-ketone carbonyl group of Chl *a* and Chl *b* and the 3-formyl carbonyl group of Chl *b* as well as the central magnesium atom. These groups are known to play an essential role in the creation of aggregates in vitro [15,16] and are factores in determining the different spectrally discrete species of chlorophyll that result on complexation with protein [17].

In the work reported here we have sought information on the mechanism of interaction between different chlorophyll molecules and LC molecules

Correspondence address: D. Wróbel, Institute of Physics, Poznań Technical University, Piotrowo 3, 60-965 Poznań, Poland

in which they have been embedded. For this purpose we have compared our present result with previous RR spectra of monomeric and aggregated forms of chlorophylls in vitro as well as of chlorophylls in vivo [10,17–21]. A separate publication will be devoted to the order parameters by using polarized RR spectroscopy [22].

2. Materials and methods

Chl *a* and Chl *b* were extracted from spinach leaves and column chromatographed on sugar powder using the method of Omata and Murata [23]. The pigments were dissolved in an LC mixture: *p*-methoxybenzylideno-*p'*-butylaniline (MBBA) and *p*-ethoxybenzylideno-*p'*-butylaniline (EBBA) (Riedel-de Haën), weight proportion 3 : 2. The samples were used without further purification. The concentration of chlorophylls in the MBBA + EBBA mixture was of the order of 10^{-2} M. Sample degradation during the experiment was avoided by degassing the solutions.

Resonance conditions for Raman spectra were obtained with 441.6 nm (He-Cd laser) and 472.7 nm (argon laser) light for Chl *a* and *b*, respectively. RR spectra were recorded at a resolution of 6 cm^{-1}, using a double-grating Raman spectrophotometer as described in refs. [10 and 17]. Summation of spectra in the 1500–1750 and 50–400 cm^{-1} regions was carried out in a multichannel analyser, RR spectra measured at room temperature were collected for disordered samples of the chlorophylls in the LC.

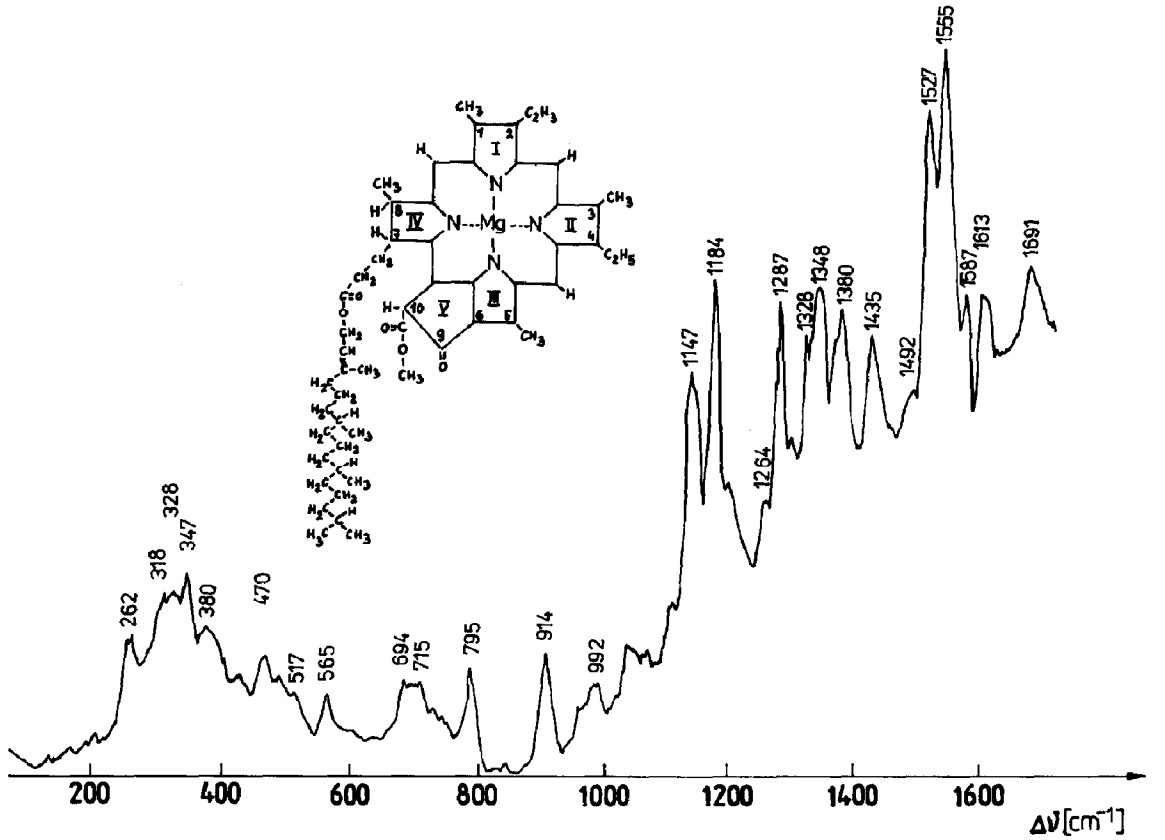

Fig. 1. RR spectrum of Chl *a* in MBBA + EBBA (after subtraction of the LC spectrum). Excitation wavelength, 441.6 nm; resolution, 6 cm^{-1}.

3. Results

3.1. RR spectra of chlorophylls

The RR spectra of Chl *a* and *b* dissolved in the MBBA + EBBA mixture and excited with light in the region of the Soret band maxima (441.6 and 472.7 nm, respectively) exhibit a complex structure with many resolved vibrational frequencies. Overall views of the RR spectra of Chl *a* and *b* in the LC are illustrated in figs. 1 and 2. The most interesting regions in these spectra are those between 1550–1700 and 50–700 cm^{-1}. These parts of the spectra provide information about the C_9 ketone (Chl *a*, Chl *b*) and C_3 aldehyde (Chl *b*) carbonyl groups, and the magnesium atom, respectively.

3.1.1. Carbonyl region (1550–1700 cm^{-1}).

The carbonyl region of the Chl *a* RR spectrum exhibits four pronounced maxima at 1555, 1587, 1613 and 1691 cm^{-1}. The major complication in the spectra of chlorophylls in the MBBA + EBBA LC matrix is the presence of a large contribution of strong bands of the LC which mask the carbonyl region (fig. 3). In particular, the MBBA + EBBA mixture yields three bands at about 1624, 1594 and 1572 cm^{-1}. The first of these is assigned to the C=N stretching vibration and latter two are involved with the benzene-ring vibrations [24–26].

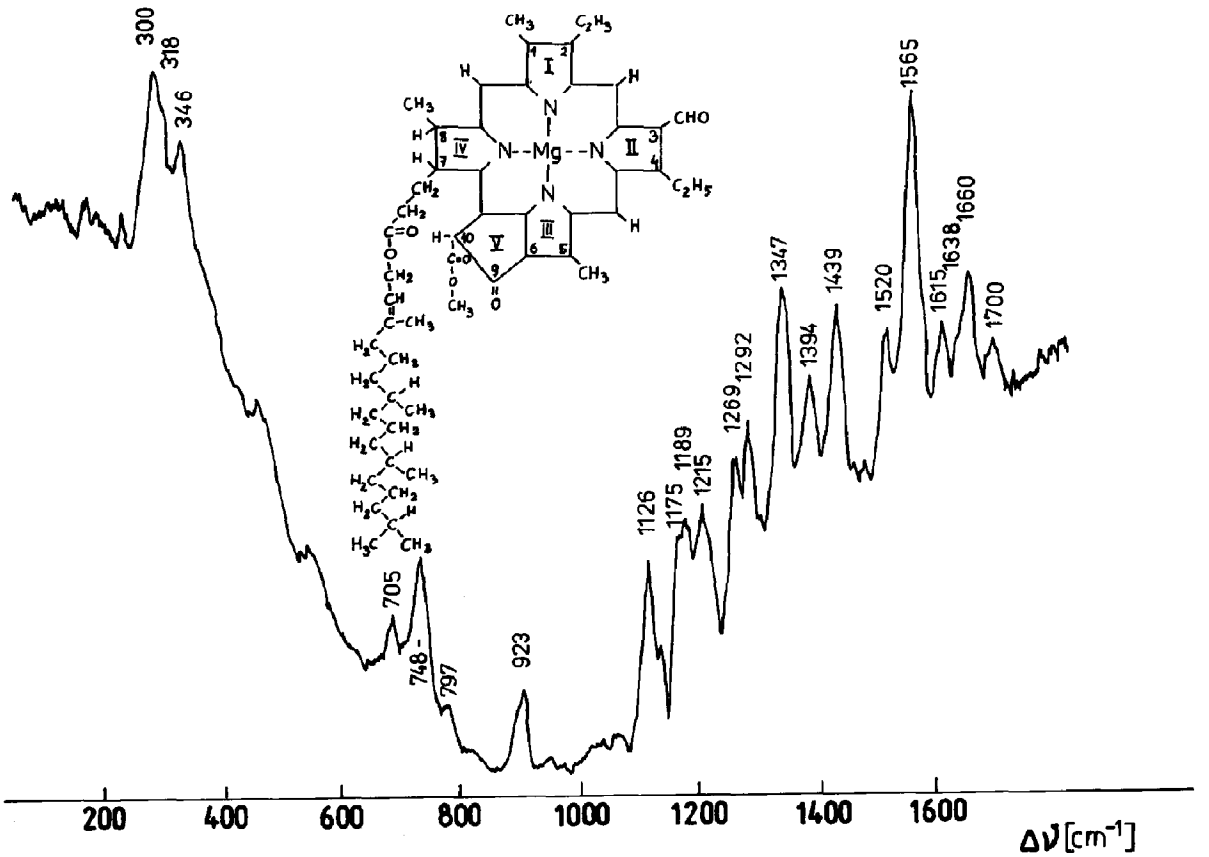

Fig. 2. RR spectrum of Chl *b* in MBBA + EBBA (after subtraction of the LC spectrum). Excitation wavelength, 472.7 nm; resolution, 6 cm^{-1}.

In the spectra of the sample pigmented with chlorophylls, maxima arising from RR scattering of the chlorophyll molecules and Raman bands of the LC matrix are observed. The LC mixture does not practically absorb in the region of Chl *a* (441.6 nm and Chl *b* (472.7 nm) absorption. The spectra of a pure MBBA + EBBA mixture are subtracted from the spectra of chlorophylls in the LC using a multichannel analyser. Thus, figs. 1 and 2 present spectra of Chl *a* and *b* respectively, in MBBA + EBBA, after subtraction of the LC spectrum.

In the region discussed one may observe some correlations between the RR spectrum of Chl *a* in polar solvent at room temperature [10,27] and Chl *a* dissolved in the LC (fig. 1). However, all of the observed frequencies in this region are downshifted with respect to Chl *a* monomer bands [10,27]. This shift may be due to the specific influence of the local electric field due to LC molecules on the chlorophyll.

It is expected that the 1691 cm^{-1} frequency arises from a stretching motion (shifted by -4 cm^{-1} with respect to the monomer) of the non-bonded 9-ketone group of Chl *a*. The 1555, 1587 and 1613 cm^{-1} bands correspond to the 1560, 1585 and 1615 cm^{-1} bands arising from C=C stretching motions of the phorbin skeleton seen in spectra of monomeric Chl *a* in vitro [10,15,27]. The band at 1527 cm^{-1} lying close to the 1555 cm^{-1} band is described in section 3. 1.2.

The carbonyl stretching region of the RR spectrum of Chl *b* in the LC is reproduced in fig. 4. This part of the Chl *b* spectrum contains a larger number of RR bands compared to that of Chl *a*. This is connected with the presence of the aldehyde group in addition to the ketone group in Chl *b* whose stretching motions are also observed in RR spectra. The stretching modes of the ketone and aldehyde groups of monomeric Chl *b* in acetone solution give rise to RR peaks at 1692 and 1661 cm^{-1}, respectively. These bands, involving the modes of non-bonded ketone and aldehyde carbonyl groups, arise from Chl *b* in the LC at 1700 and 1660 cm^{-1}, respectively. However, the band peaking at 1700 cm^{-1} is broad. It involves

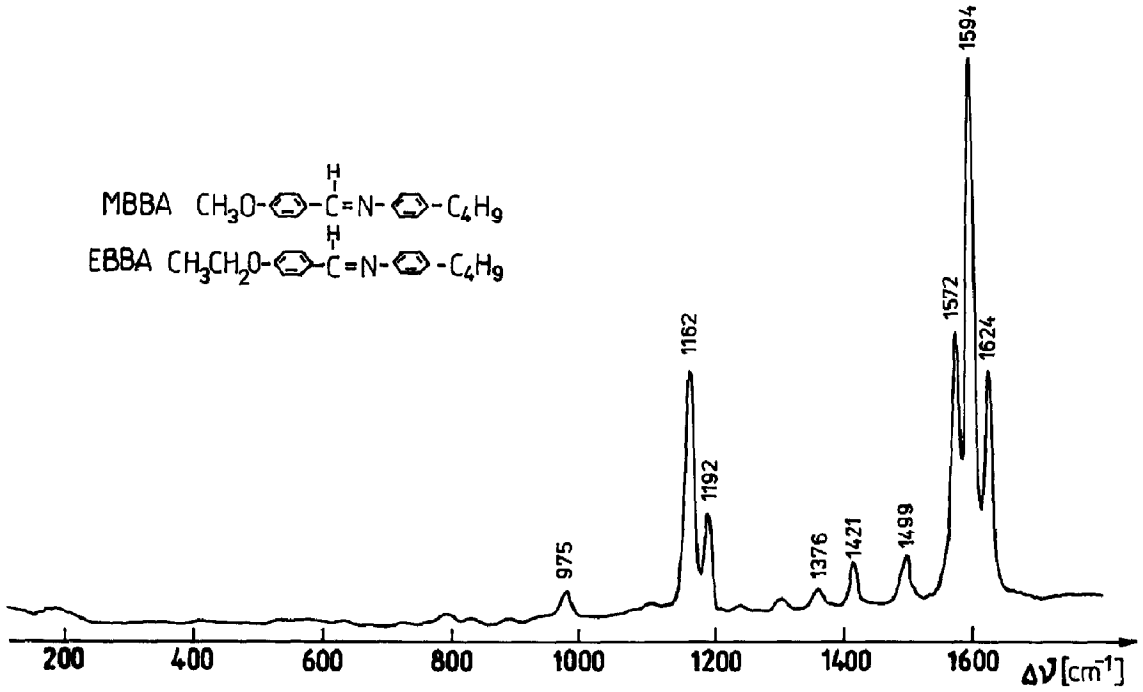

Fig. 3. Raman spectrum of MBBA + EBBA mixture. Excitation and resolution as in fig. 1.

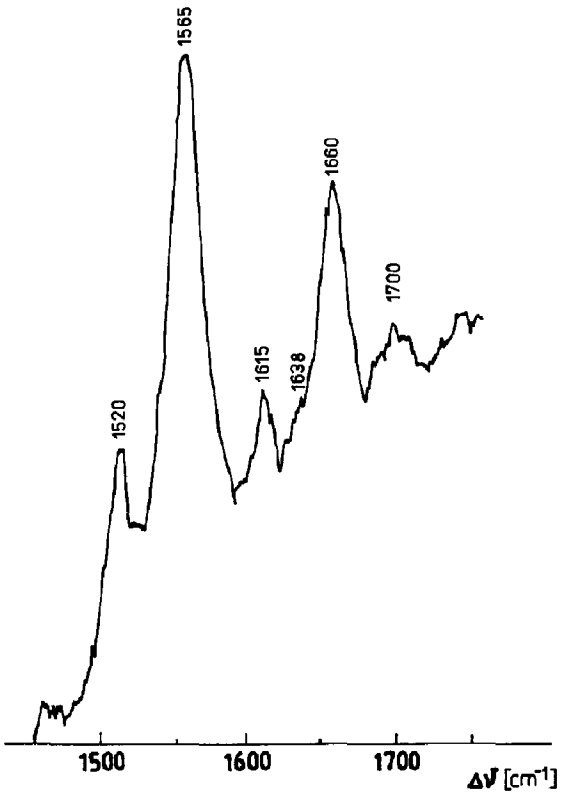

Fig. 4. Carbonyl stretching region of Chl *b* in MBBA + EBBA. Averaged by summation. Excitation and resolution as in fig. 2.

the 9-carbonyl group, either free or weakly interacting with the environment. Also, the very small contribution at 1638 cm^{-1} appears to exist on the lower wavelength side of the 1660 cm^{-1} band. The peak at 1615 cm^{-1} with the weak feature at about 1621 cm^{-1} is due to C = C bond stretching of the methine bridges whereas the strong band at 1565 cm^{-1} (1562 cm^{-1} in acetone solution) is assigned to monomeric Chl *b* [10,17,27]. A medium-strength band at 1703 cm^{-1} characteristic of the 9-carbonyl mode is found in the RR spectra of free pheophytin *a* (data not shown). The rest of the spectrum does not differ from that of isolated pheophytin *a* [10,27].

3.1.2. Skeleton vibrations (700–1550 cm^{-1})

The bands in this part of the spectra arise from in-plane stretching and angular modes involving C=C and C=N bonds in the tetrapyrrole ring, some being very sensitive to the formation aggregates between chlorophyll molecules.

Comparing the spectra of Chl *a* in the LC (fig. 1) with those previously obtained for Chl *a* in acetone solution [10,17,27], one can find bands which match with the RR frequencies of the latter. For instance, there are the strong bands at 1527, 1287 and 1184 cm^{-1}, the medium-strength band at 1348 cm^{-1}, and others. Among the many bands in this part of the spectrum there are some which are shifted only about 2–3 cm^{-1} with respect to those of monomeric Chl *a* [10,17,27]. The small differences compared with the spectrum of monomeric Chl *a* in acetone can be related to the electronic interaction between chlorophyll and the LC molecules. On the other hand, $(Chl\ a)_n$ oligomers are known to exhibit characteristic down-shifts of some bands with respect to those of the monomers. Thus, for example, the 1527 cm^{-1} band is shifted by -8 cm^{-1}, that of 1287 cm^{-1} by -4 cm^{-1} and that of 1264 cm^{-1} by -5 cm^{-1}. However, such large changes are not observed for Chl *a* in the LC. If oligomers existed, the bands at 1287, 992 and 750 cm^{-1} would be more strongly enhanced with respect to the other bands of this region. Moreover, the 694 cm^{-1} band should be decreased in intensity. Thus, it is seen that the bands which are present in the 700–1550 cm^{-1} region of the spectrum do not coincide closely with those of self-associated Chl *a*. Therefore, it allows us to suppose that essentially only monomeric Chl *a* exists in the LC samples investigated.

Very similar features can be found for Chl *b* in the LC (fig. 2). These include strong bands at 1439, 1347, 1292, 1269, 923 and 748 cm^{-1}, although the relative intensities of some are not maintained (for instance, the 1394 cm^{-1} band is too small compared to the 1439 cm^{-1} band). The bands mentioned above are particular sensitive to oligomer formation. The observed intensity ratios indicate that for Chl *b*, small amounts of oligomers exist in the LC, but there are some bands belonging to the carbonyl region (1700, 1660 and 1565 cm^{-1}) attributed to free Chl *b* molecules.

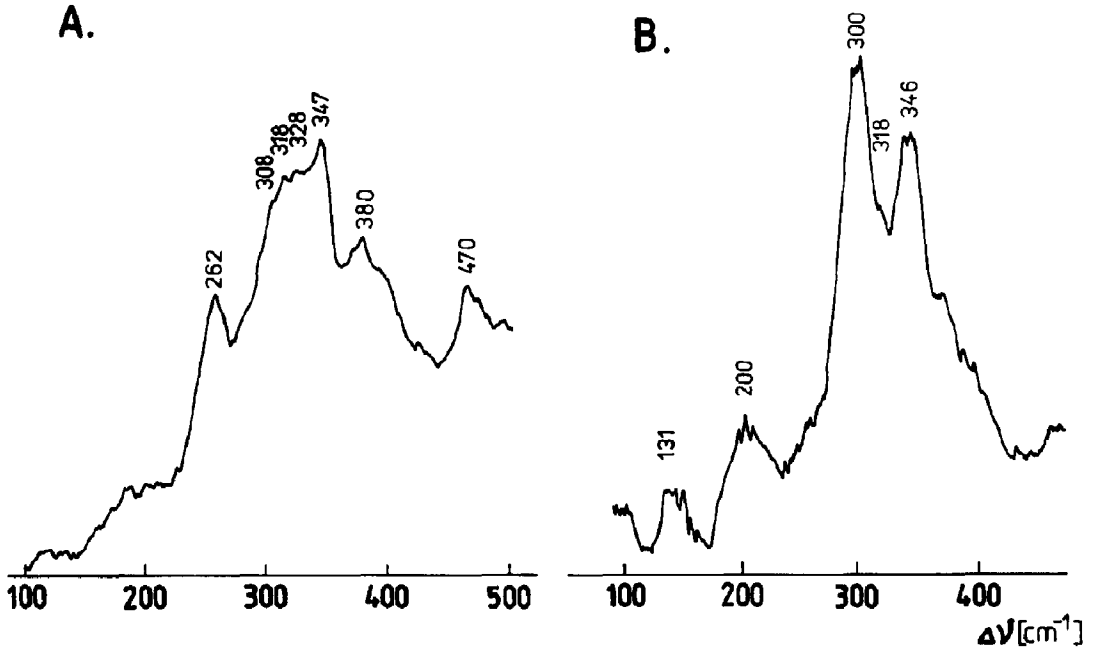

Fig. 5. RR spectra, 50–400 cm^{-1}. (A) Chl *a* in MBBA + EBBA, excitation wavelength 441.6 nm, (B) Chl *b* in MBBA + EBBA, excitation wavelength 472.7 nm. Resolution 6 cm^{-1}.

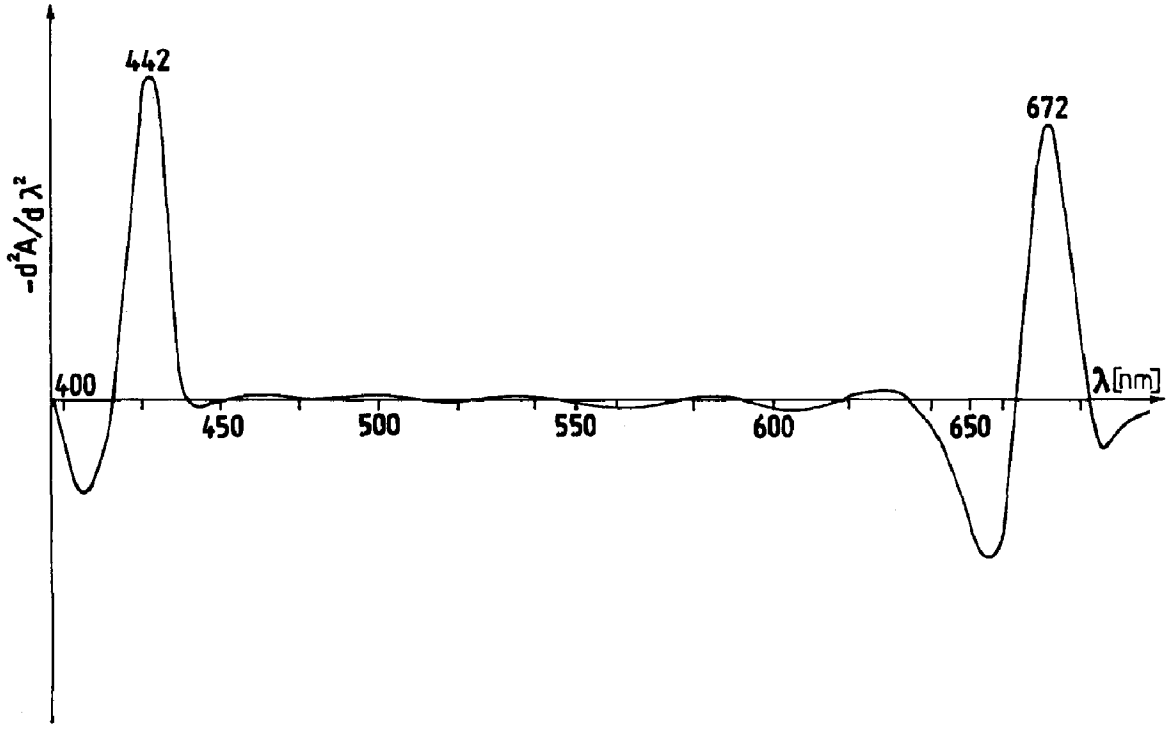

Fig. 6. The second derivative absorption spectrum of Chl *a* in MBBA + EBBA.

3.1.3. 50–700 cm^{-1} region

Fortunately, the bands in this region are not disturbed by the LC frequencies. The frequencies observed arise from planar deformation of the phorbin skeleton and vibrational coupling between motions of the Mg-N_4 grouping [10,17,27]. The results obtained for Chl *a* and *b* in MBBA + EBBA on excitation at 441.6 and 472.7 nm, respectively, are reproduced in fig. 5 (and figs. 1 and 2).

Some of the bands are present in the RR spectra of chlorophylls in polar solvents and correspond to modes active in chlorophylls in their monomeric state. Nevertheless, for Chl *a* (fig. 5A) changes in the relative intensities and positions of bands are observed. The most intense band at 347 cm^{-1}, shifted about -5 cm^{-1} with respect to that of monomeric Chl *a* in acetone [10,17,27], is thought to be characteristic of modes involving motions of the magnesium atom as well as of the nitrogen atoms. We have found the same result for the 318 cm^{-1} band (shifted by -3 cm^{-1}) which is not as sharp as the previous one. In addition, a small 308 cm^{-1} shoulder and a weak band at 328 cm^{-1} are observed. The band at 262 cm^{-1} is sharp as in the case of monomeric Chl *a* [10,17,27], although it is less intense. Moreover, the RR spectrum of Chl *a* dissolved in the LC differs from those in acetone solution in the presence of a pronounced band at 380 cm^{-1}, which is only weak in the monomer spectrum [10,17,27]. The bands at 470, 565 and 694 cm^{-1} may correspond to monomer bands.

The 50–700 cm^{-1} region of the RR spectrum of Chl *b* is depicted in fig. 5B and is of a similar character to that of Chl *a*. In the case of Chl *a* the RR bands may be divided into two groups. The first exhibits maxima characteristic of the monomer state, and the second, those indicative of interaction with an undefined partner. For Chl *b* in the LC the band at about 300 cm^{-1} is broad with respect to that of the monomer. Its halfwidth is about 30 cm^{-1}, while that of the monomer is smaller than 25 cm^{-1}. Furthermore the small shoulder around 318 cm^{-1} is on the long-wavelength side of this band. The other frequencies observed correspond to those for the Chl *b* monomer.

3.2. Electronic absorption spectra

The absorption spectra of chlorophylls in the LC show changes in the relative intensities of the bands and also in their position by 10–20 nm compared to those observed, for instance, in diethyl ether [3–6]. The derivative absorption (DA) spectrum of Chl *a* was also measured in order to confirm the interpretation of the RR studies. The DA spectrum of Chl *a*, as an example, in MBBA + EBBA (fig. 6) shows a narrow band at 672 nm and no shoulder is found, thus providing evidence for the absence of aggregated Chl *a* in the LC sample investigated [28].

4. Discussion

The present results allow some conclusions to be drawn as to the nature of bonding interactions between the chlorophyll molecules investigated and their host solvent. The characteristic of the carbonyl region of the RR spectrum of Chl *a* in the LC strongly suggests that the 9-ketone groups, vibrating at 1691 cm^{-1}, are essentially free from intermolecular bonding. The RR spectra of $(Chl\ a)_n$ oligomers in various solvents show that 9-ketone groups bound to the magnesium atom of another Chl *a* molecule have a stretching frequency near 1650 cm^{-1} [19,20]. However, this band, attributed to oligomers, is found to be absent in the RR spectra of Chl *a* dissolved in the LC. Our conclusion concerning the monomeric state of Chl *a* in the LC is further confirmed by examination of some spectral features of the skeletal vibration region (700–1550 cm^{-1}). This region is known to be very sensitive to oligomer formation not only because of structural changes involving the vibrational groups but also because of variations in the π-electron interactions. The oligomerization affects the following pairs of bands: 1348–1334, 1290–1185, 1226–1207, 1157–1147, 759–695 and 720–695 cm^{-1} (the 1226 cm^{-1} band arises only for $(Chl\ a)_n$ oligomers [27]). In particular, one can observe variations of the intensity ratios of these bands as a consequence of the presence of oligomer. These intensity ratios were compared with the data given in

ref. 27. The majority of these ratios support our first suggestion concerning the state of Chl *a* that there are no oligomers of Chl *a* or only very few in the LC sample. Nevertheless, Subramanian et al. [29] could not exclude the association of aggregate formation among Chl *a* molecules in dodecylcyanobiphenyl. Additional confirmation is found in the derivative absorption spectra. Previously reported CD spectra of Chl *a* in the LC also indicated that this pigment is at least predominantly in its monomeric state [7,8]. Chl *a* in its monomeric form in solution is necessarily bound to solvent molecules [30]. The magnesium atom in Chl *a* when dissolved in a polar solvent is thus most probably bound to two solvent molecules [10,31,32], so that this atom is in a hexacoordinated state and no 310 cm^{-1} band is evident in the RR spectra of monomeric Chl *a*. The number of ligands on the Mg atom of Chl *a* in the LC cannot be definitively ascertained from the RR spectrum because of the specious signal at about 328 cm^{-1}. The low-frequency band set (50–400 cm^{-1}) help only tentatively in indicating the probable number of ligands. Thus, the band at 318 cm^{-1} is broader than that observed for typical monomeric Chl *a* and exhibits a shoulder near 308 cm^{-1}. A 308 cm^{-1} component would be observed if only one extramolecular ligand were bound to the magnesium, i.e., it indicates pentacoordination of the magnesium atoms of Chl *a* molecules in the LC. These fifth ligands on the magnesium may be LC molecules in view of the electron-donor properties of the latter [30] (or maybe a small trace of coexisting water). The nitrogen atoms of the LC molecules have an available lone pair of electrons which may be donated in bonding magnesium atoms.

In the RR spectra of Chl *b*, we attributed the 1700 and 1660 cm^{-1} frequencies to the stretching modes of the 9-ketone and 3-aldehyde groups, respectively. We are thus led to the same conclusion as for Chl *a*: both ketone and aldehyde groups are free from intermolecular bonding. This result permits us to deduce that the majority of Chl *b* molecules are in the monomeric form. However, the weak component at 1638 cm^{-1} would possibly indicate that a small number of Chl *b* are bonded through their aldehyde groups. Moreover, some of the characteristic intensities of bands in the skeletal region are intermediate between those observed for monomers and oligomers [27]. These values would indicate intermolecular interaction with MBBA + EBBA and/or other Chl *b* molecules but the 1660 cm^{-1} value definitively indicates a free vibrator. The frequencies at 1439 and 1565 cm^{-1} are those of monomeric Chl *b* and additionally support the conclusion that most Chl *b* molecules are monomers with free aldehyde and ketone carbonyls. It is generally accepted that the magnesium atom of chlorophyll assumes a pentacoordinated rather than hexacoordinated state [30,33–35]. The question of the coordination behaviour of Chl *b* in the LC can be resolved using the low-frequency sets of RR spectra. The bands at 305–320 cm^{-1} are known to be sensitive to the number of ligands bound to the magnesium [10,18,20]. The band at about 300 cm^{-1} of Chl *b* in the LC is broad with a halfwidth which is increased with respect to monomeric Chl *b*. This feature and the shoulder around 318 cm^{-1} indicate that many Chl *b* molecules have pentacoordinated magnesium atoms. This result has also been confirmed by Fujiwara and Tasumi [35]. The single external ligand may be the LC molecule as in the case of Chl *a*.

Nevertheless, Frackowiak et al. [7,8,36,37] have observed different spectral properties of Chl *a* and *b* in MBBA + EBBA mixture. The CD signals previously observed for Chl *a* and *b* are opposite to each other [7,8]. Also, the dielectric properties of Chl *a* and *b* solutions in the same nematic solvent have been found to be different [36], as have the linear dichroic ratios [37], suggesting that the LC must interact differently with both Chl *a* and *b*. The only indication that we have from RR data that Chl *a* and *b* may behave differently in the LC is from the relative band intensities in the skeletal regions. These suggest that Chl *b* may experience a stronger interaction with the LC molecules than does Chl *a*, but this problem seems to remain open.

The present RR results provide evidence that:

(1) most Chl *a* and *b* species are monomeric even at the high concentration used,

(2) the magnesium atoms in both Chl *a* and *b* are pentacoordinated.

Acknowledgements

The author wishes to thank the Department of Biophysics at the Centre for Nuclear Research in Saclay for a Fellowship and acknowledges financial support under Project no. RP BP II 11.4.2 coordinated by Łodź University. The measurements of RR spectra were made at the C.N.R. in Saclay with the helpful advice of Dr. Marc Lutz. The author also gratefully acknowledges fruitful discussion with him. The technology for preparation of samples was developed and the samples were made in the Institute of Physics at the Technical University in Poznań.

References

1 J. Breton and A. Vermeglio, in: Photosynthesis: energy convertion by plants and bacteria, ed. Govindjee (Academic Press, New York, 1982) vol. 1, p. 153.
2 R. Journeaux and R. Viovy, Photochem. Photobiol. 28 (1978) 243.
3 Z. Salamon and T. Martyński, Biophys. Chem. 9 (1979) 369.
4 D. Frąckowiak, D. Bauman, H. Manikowski and T. Martyński, Biophys. Chem. 6 (1977) 369.
5 D. Frąckowiak, Acta Phys. Polon. A54 (1978) 757.
6 D. Bauman and D. Wróbel, Biophys. Chem. 12 (1980) 83.
7 D. Frąckowiak, D. Bauman and M.J. Stillman, Biochim. Biophys. Acta 681 (1982) 273.
8 D. Frąckowiak, D. Bauman, M.J. Stillman and H. Manikowski, in preparation.
9 D. Frąckowiak, J. Szurkowski, S. Hotchandani and R.M. Leblanc, Mol. Cryst. Liq. Cryst. 111 (1984) 359.
10 M. Lutz, J. Raman Spectrosc. 2 (1974) 497.
11 B. Cartlig and R. Wilbrandt, Biochim, Biophys. Acta 637 (1981) 61.
12 M. Lutz, L. Chinsky and P.Y. Turpin, Photochem. Photobiol. 36 (1982) 503.
13 R. Schiffmiller, R.H. Callender, W.H. Waddell, R. Govindjee, T.G. Ebrey, H. Kakitani, B. Honig and K. Nakanishi, Photochem. Photobiol. 41 (1985) 563.
14 R. Wilbrandt, N.-H. Jensen and C. Houée-Levin, Photochem. Photobiol. 41 (1985) 175.
15 J.J. Katz, R.C. Dougherty and L.J. Bouchter, in: The chlorophylls, eds. L.P. Vernon, G.R. Seely (Academic Press, New York, 1966) p. 186.
16 K. Ballschmiter and J.J. Katz, Biochim. Biophys. Acta 256 (1972) 307.
17 M. Lutz, Biochim. Biophys. Acta 460 (1977) 408.
18 M. Lutz, J. Kléo, R. Gilet, M. Henry, R. Plus and J. Leickman, in: Proceedings of the 2nd International Conference on Stable Isotopes, eds. E.R. Klein and P.D. Klein (U.S. Department of Commerce, Springfield, VA, 1975) p. 462.
19 M. Lutz and J. Breton, Biochim. Biophys. Res. Commun. 53 (1973) 413.
20 M. Lutz, in: Lasers in physical chemistry and biophysics, ed. J. Joussot-Dubien (Elsevier, Amsterdam, 1975) p. 451.
21 M. Lutz, J.S. Brown and R. Rémy, in: Chlorophyll organization and energy transfer in photosynthesis (Elsevier/North-Holland 1979) p. 105.
22 D. Wróbel, in preparation.
23 T. Omata and N. Murata, Photochem. Photobiol. 31 (1980) 183.
24 G. Vergoten and G. Fleury, Mol. Cryst. Liquid Cryst. 30 (1975) 213.
25 G. Vergoten and G. Fleury, Mol. Cryst. Liquid Cryst. 36 (1976) 36.
26 B.J. Bulkin, in: Advances in liquid crystals, ed. G.H. Brown (Academic Press, New York, 1976) vol. 2, p. 199.
27 M. Lutz, Ph.D. Thesis, P.&M. Curie University, Paris, France (1979).
28 D. Wróbel, Photosynthetica 11 (1977) 90.
29 R. Subramanian, L.K. Patterson and H. Levanon, Photochem. Photobiol. 41 (1985) 511.
30 I. Renge and R. Avarmaa, Photochem. Photobiol. 42 (1985) 253.
31 C.B. Storn, A.H. Corwin, R.R. Arellano, M. Martz and R. Weintraub, J. Am. Chem. Soc. 88 (1966) 2525.
32 J.J. Katz and J.R. Noriss, Jr, Curr. Top. Bioenerg. 5 (1973) 41.
33 J.J. Katz, W. Oettmeier and J.R. Norris, Phil. Trans. Roy. Soc. Lond. B 273 (1976) 227.
34 H.-S. Chow, R. Serlin and C.E. Strouse, J. Am. Chem. Soc. 97 (1975) 7230.
35 M. Fujiwara and M. Tasumi, J. Phys. Chem. 90 (1986) 250.
36 D. Frąckowiak, S. Hotchandani and R.M. Leblanc, Photobiochem. Photobiophys. 7 (1984) 41.
37 D. Frąckowiak, S. Hotchandani and R.M. Leblanc, Photobiochem. Photobiophys. 6 (1983) 339.