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Circular dichroism and magnetic circular dichroism spectra of chlorophylls *a* and *b* in nematic liquid crystals

II. Magnetic circular dichroism spectra

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Absorption and magnetic circular dichroism (MCD) spectra are reported for chlorophyll (Chl) *a* and Chl *b* dissolved in nematic liquid crystal solvents. The spectra were measured with the dye molecules oriented uniaxially along the direction of the magnetic field and measuring light beam. It is significant that under such conditions the MCD spectra recorded in the wavelength region of the Q and Soret bands of the chlorophyll are essentially unchanged with respect to rotation of the sample cell around this axis, even though there is almost complete orientation of the chlorophyll molecules by the liquid crystals. The MCD spectra of Chl *a* and *b* in the nematic liquid crystal solvents used in this study are surprisingly similar to the spectra obtained under isotropic conditions. These results illustrate an important technique with which to examine the optical spectra of dyes oriented in liquid crystal matrices in which the anisotropic effects can be reduced the negligible proportions by the application of a strong magnetic field parallel to the direction of the measuring light beam. The first deconvolution calculations are reported that describe the deconvolution of pairs of absorption and MCD spectra, in the Q and B band regions, for both Chl *a* and *b*. The spectral analysis to obtain quantitative estimates of transition energies was accomplished by carrying out detailed deconvolution calculations in which the both the absorption and MCD spectral envelopes were fitted with the same number of components; each pair of components had the same band centres and bandwidth values. This procedure resulted in an assignment of each of the main transitions in the absorption spectra of both Chl *a* and *b*. Chl *a* is clearly monomeric, with Q_y, Q_x, B_y and B_x located at 671, 582, 439 and 431 nm, respectively. Analysis of the spectral data for Chl *b* located Q_y, B_y and B_x at 662, 476 and 464 nm, respectively.

1. Introduction

Spectroscopic studies of a variety of different chlorophylls dissolved in organic solvents, in various rigid media and in chloroplasts have provided a wealth of information about the role of chlorophyll in photosynthesis [1–22].

In particular, analysis of the intense and distinctive absorption and magnetic circular dichroism (MCD) spectra of the chlorins and chlorophylls in these media has provided considerable information concerning the relative splitting of the excited states [1,2,3,6,11,12] and the effects of aggregation and peripheral ring substituents on the energies and polarizations of the transitions that lie in the visible region [1,3,8–12,16]. Assignments of the optical spectra of a variety of chlorophylls and chlorins have been carried out, in the main, by matching the transition energy and the polarization of each transition to theoretical predictions [1,2,6,10–12,15,17,18,22,24,26]. However, in many cases this is considerably more difficult than for the analogous porphyrin spectra because there the bands overlap so extensively, especially in the Soret region [1].

Because it is the role of chlorophylls in the

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photosynthetic process that is of prime interest to us, spectral information (e.g., transition energies and polarizations) obtained from optical data recorded from samples oriented in vitro will best model the spectra observed from chlorophylls located in chloroplasts [11,16,27–29]. While several different chlorophylls are found in photosynthesizing organisms, chlorophyll (Chl) *a* and *b* play the most important roles in higher plants [16,27–29]. Measurement of optical spectra using polarized light provides considerable information if the chromophore is oriented in a known manner [16]. Experiments in such a vein include measuring absorption and fluorescence spectra from chlorophylls as monolayers [19–21], within a single crystal of a pyrochlorophyllide *a*-apomyoglobin complex [13], or in liquid crystal matrices [23]. Liquid crystal matrices, in particular, offer a matrix that will provide order for a molecule that is dissolved in the bulk solvent. In addition to the initial orientation properties of the liquid crystal molecule, the orientation can be readily changed by applying electric or magnetic fields. We have studied previously in some detail the polarized optical absorption and circular dichroism (CD) spectra of chlorophyll molecules dissolved in nematic liquid crystals [14,30–32], and have suggested that this type of system is also useful in modelling the in vivo environment of the chlorophylls in chloroplasts. In a preliminary paper [33], we investigated the sensitivity of MCD spectroscopy in detecting changes in the electronic spectrum when electron acceptors are added to solutions of Chl *a* in a nematic liquid crystal matrix.

The MCD technique has also been shown to be able to provide considerable information about the electronic structure of the porphyrin π ring, as the MCD spectrum is sensitive to the polarization properties of each transition [3,11,12,34–38]. MCD spectra of the symmetric (D_{4h}) porphyrins and phthalocyanines have been widely published (examples include, refs. 37–40). The MCD spectra of such molecules are dominated by Faraday *A* terms [11]. The determination of the energies and signs of the *A* terms provides valuable information to aid in both the assignments of individual bands and in the calculations of the electronic configurations of the excited states involved [40,41]. In general, these data have been reported for solutions at room temperature, although spectra from molecules deposited as thin films [42], dissolved in polymers and deposited in inert matrices [39] have also appeared.

The chlorophylls have attracted similar attention, and several papers describing MCD spectra of both chlorophylls and chlorins have been published [1–6,11,12,24]. While the chlorophyll ring bears close resemblance to the porphyrin ring, the additional five-membered ring attached to pyrrole III and the reduction of pyrrole IV serve to reduce the symmetry from the D_{4h} of many prophyrins. The MCD spectra are, therefore, expected to show only *B* terms (Gaussian-shaped envelopes centred on the absorption band and of either positive or negative sign, where the intensity in these *B* terms arises from mixing between excited states of *x* and *y* polarization [1,10–12,35,36]). The signs of the MCD intensity of the major components in the visible region of chlorophylls depend largely on the relative splitting energies of the highest occupied molecular orbitals (HOMOs) and lowest unoccupied molecular orbitals (LUMOs) [10–12]. While Q_x and Q_y are usually clearly discernable in the MCD spectrum of chlorophylls because of the distinct MCD intensity of opposite sign, the B_x and B_y bands overlap significantly and thus an unambiguous assignment is not always possible [1–3,6,10].

For our present study, we are concerned with spectral data for chlorophyll recorded for ordered molecules. Amongst the published MCD data for the chlorophylls, four reports involve Chl *a* [1–4], while two describe spectra for Chl *b* [5,6]. With the exception of the work of Bolton et al. [4], the MCD data reported have been measured from chlorophylls dissolved in organic solvents, i.e., using isotropic samples.

We describe, in this paper, the first detailed MCD study of Chl *a* and Chl *b* dissolved in nematic liquid crystal solvent matrices. In these experiments, the magnetic field was applied perpendicularly to the windows of the cell, i.e., parallel to the measuring light beam. At the high magnetic fields used for these MCD measurements, we find that the chlorophyll molecules are oriented

uniaxially along the axis parallel to the magnetic field. Under such conditions, we find that there are no significant textural effects in the MCD spectra of the chlorophyll, even though such matrices are still completely anisotropic. As a result of this, we are able to obtain MCD spectra that are unperturbed by polarization effects unrelated to the magnetic moments of the chlorophyll's excited states. The MCD data described here closely resemble those measured from randomly distributed chlorophylls. Detailed deconvolution calculations were carried out on these data and, by using the strong magnetic moments observed in the MCD spectra under the $Q_{x/y}$ (0-0) and $B_{x/y}$ (0-0) bands, we are able to suggest assignments for the absorption spectra of both Chl *a* and Chl *b* in the liquid crystal matrices.

2. Materials and methods

Solutions of chromatographically purified chlorophylls of concentration about 10^{-3} M were prepared in the following liquid crystal solvents: (1) *p*-methoxybenzylideno-*p*-butylaniline (MBBA) + *p*-ethoxybenzylideno-*p*-butylaniline (EBBA); (2) MBBA + EBBA + 4-dimethylaminobenzonitryl (DAB); and (3) *p*-pentyl-*p*-cyanobiphenyl (PCB). The solutions of the chlorophyll molecules dissolved in the liquid crystal matrices were studied in specially constructed optical cells of 20 μm thickness [43]. As a result of the special electrode preparation [14], the liquid crystal molecules are aligned almost uniaxially within the cell, but are tilted at an angle of 20° with respect to the plane of the windows. Because there is a strong interaction between molecules of the liquid crystal solvent molecules and those of the chlorophyll, the pigment molecules orient similarly to the liquid crystal solvent molecules [23].

Absorption spectra were recorded using a Cary 219 spectrophotometer. A cell filled with pure liquid crystal solvent was used as a reference sample. MCD experiments were carried out at room temperature using a CD spectrometer constructed by W.R. Browett and M.J. Stillman at the University of Western Ontario (unpublished data). An Oxford Instruments (Oxford, U.K.) SM2 superconducting magnet provided a variable magnetic field for MCD experiments up to a maximum of 5.5 T. MCD signal intensities were calibrated by measuring the negative signal at 505 nm observed for aqueous $CoSO_4$ solutions. In our case, a value for $[\theta]_M$ of -62.0 degree cm^2 dmol^{-1} T^{-1} was measured, or in terms of $\Delta\epsilon_M$, -1.88×10^{-2} l mol^{-1} cm^{-1} T^{-1}. With the exception of field dependence studies, all the MCD spectra presented here were measured using a 5.5 T magnetic field. Under these conditions, the liquid crystal molecules are oriented almost perfectly in the direction of the magnetic field (H), which is perpendicular to the plane of the windows in the sample cell. We have shown previously [14] that the linear dichroism is very low in such an arrangement.

In all our measurements we chose an orientation of the cell that resulted in a minimum contribution to the measured CD spectrum by the texture of the liquid crystal solvent. For a cell filled with the MBBA + EBBA mixture (with and without DAB), this was with a horizontal alignment of the initial, liquid crystal orientation axis (measured before the magnet was switched on). For PCB we found that a vertical position of this axis was optimal. The dependence of the MCD spectrum on cell position is caused by the fraction of liquid crystal molecules that are located near the surface of the cell windows. The molecules will interact strongly with the orienting layer of SiO_x. Such molecules may retain their initial orientation even at high magnetic field intensities.

Deconvolution calculations were carried out using the program SIMPFIT [44] on an IBM S9001 computer. The data were organized and plotted with the spectral database program, Spectra Manager [45].

3. Results and discussion

3.1. Nomenclature

In the chlorophylls, the absorption spectrum observed from the near ultraviolet to the near-infrared arises primarily from transitions to the first two singlet electronic states of the porphyrin π

ring [1,11,12,18]. Both of these states are split from the initial E_u degeneracy found for D_{4h} porphyrin geometry by the coupling of ring V [1,11,12,18,25,26,39]. The resultant absorption falls into two bands which are labelled Q and B (or Soret), for the red and blue regions of the spectrum, respectively [1,11,12,17,39]. Each band may comprise several transitions, these being a mixture of vibrational bands built on the x- and y-components of the pure electronic transitions that result from the loss of degeneracy as the symmetry is lowered from the D_{4h} of a symmetric porphyrin [10]. The individual bands in the absorption spectrum of chlorophylls are polarized approximately along the x- and y-directions of the chlorin ring, and are known as Q_y, Q_x, B_y and B_x [1,10–12]. For molecular species of low symmetry, all states will be non-degenerate, and only B terms will be observed in the MCD spectrum [1,35,36]. The signs of the MCD B terms will depend on the ordering of the previously degenerate excited states [10], on the peripheral substituents of the porphyrin ring [2,3,11,12] and on the precise mixture of states that couple under the influence of the applied magnetic field and allow the MCD intensity to be observed [36]. For Chl a, alternation of the signs of the B terms in the Q band region has been used to aid in the identification of the lowest energy Q_y(0-0) transition (positive intensity), and then, from amongst several other bands, the Q_x(0-0), from its strong negative MCD intensity [1,2,6,11,12,24].

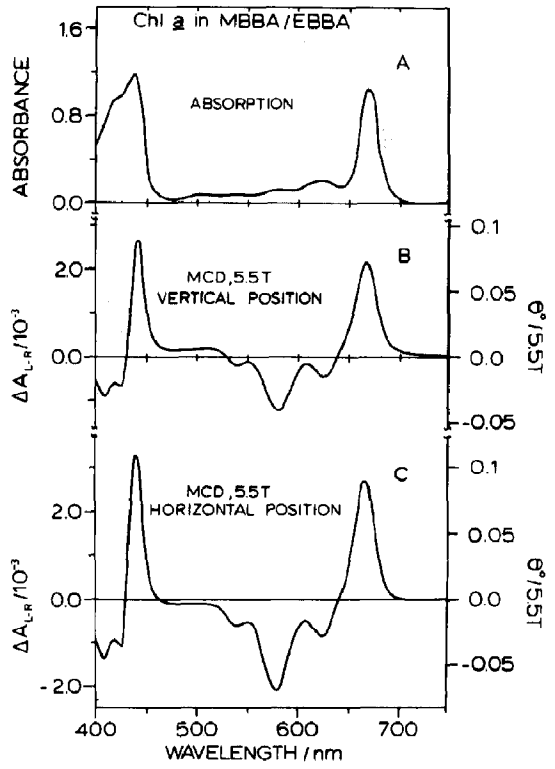

Fig. 1. Absorption and MCD spectra of Chl a dissolved in an MBBA + EBBA liquid crystal mixture, $c = 7 \times 10^{-3}$ mol l^{-1}. (A) Absorption spectrum. (B and C) MCD at 5.5 T, recorded with vertical (B) and horizontal (C) positions of the initial, zero-field liquid crystal orientation axis. The units for the MCD signal intensity are ΔA and have not been normalized for magnetic field strength.

3.2. *Spectra of Chl a and b in the liquid crystal matrices*

Fig. 1 shows the absorption and MCD spectra recorded for Chl a in the MBBA + EBBA liquid crystal mixture. The MBBA + EBBA mixture has a negative dielectric anisotropy (i.e., $\Delta\epsilon < 0$) and a positive magnetic anisotropy (i.e., $\Delta\mu > 0$). MCD spectra of Chl a in the MBBA + EBBA solvent are shown for two mutually perpendicular positions of the initial (at zero field) liquid crystal orientation axes [14], which are obtained by rotating the cell around the direction of the magnetic field. Surprisingly, the differences between these two spectra are very small, resulting primarily from differences in the positions of the baselines. The absorption spectrum is dominated by a band near 670 nm (the Q band region) and a split band near 430 nm (the Soret or B band region). The MCD spectrum shows several bands of alternating sign. The lowest energy band (at 666 nm) has positive MCD intensity and is associated with the resolved absorption at 670 nm, the other MCD bands in the visible region being associated with less well-resolved features in the absorption spectrum. The MCD bands in the Soret region reflect the same positive-to-negative sign alternation seen under the Q band, the positive band lying under the main absorption peak at 430 nm. The band

Table 1

Band maxima (nm) in the absorption and MCD spectra of Chl *a* and *b*, obtained directly from the spectral data

Absorption spectra				MCD spectral features	
This work		Hoff [a]	Weiss [b]	This work	Weiss [b]
Chl *a*					
670	$Q_y(00)$	680	661 $Q_y(00)$	666(+)	662(+)
622		625	615	626(−)	618(−)
584	$Q_x(00)$	587	575 $Q_x(00)$	582(−)	575(−)
539			530	539(−)	
506					
436	$B_y(00)$	430	428 $B_x(00)$	440(+)	439(+)
	$B_x(00)$		$B_y(00)$		
420		420		427(−)	420(−)
			409		
				408(−)	398(−)
Chl *b*					
662	$Q_y(00)$	650		662(+)	
611		600		624(−)	
572				592(−)	
547				566(−)	
				539(−)	
469	$B_y(00)$	465		481(+)	
	$B_x(00)$				
				458(−)	
420		430		435(−)	
				414(−)	

[a] Ref. 21, for Chl *a* and *b* in lecithin multilayers.
[b] Ref. 1, for Chl *a* in diethyl ether.

maxima obtained directly from these spectra are listed in table 1 and are compared with data previously published by Weiss [1] and Hoff [21].

Fig. 2 shows absorption and MCD spectral data measured for Chl *b*, in the MBBA + EBBA + DAB liquid crystal mixture, where $\Delta\epsilon > 0$ and also $\Delta\mu > 0$. Very similar spectra were also observed when Chl *b* was dissolved in MBBA + EBBA, in the absence of DAB (where $\Delta\epsilon < 0$), and also in PCB (where $\Delta\epsilon > 0$). Thus, it appears that the dielectric anisotropy ($\Delta\epsilon$) of the liquid crystal host has little influence on the observed MCD spectrum of Chl *b*. The absorption spectrum is dominated by two main band envelopes, at 660 nm (Q) and 460 nm (Soret or B). The lowest energy positive band in the MCD spectrum (at 633 nm) lies under the 660 nm absorption component, a strong negative band located at 624 nm being below a weak absorption band. In the Soret region, two MCD bands of opposite sign lie under the unresolved absorption band envelope. As with the Chl *a* sample, two perpendicular orientations of the cell were again used to obtain the MCD spectra which are shown in fig. 2B and C, respectively. We find practically no change in relative band intensities between the two spectra; the spectra are almost superimposable. Band maxima measured directly from the spectral data are listed in table 1.

3.3. Effect of orientation of the chlorophyll by the liquid crystal solvent on the MCD spectrum

The MCD spectra reported in this work for Chl *a* and *b* dissolved in liquid crystals are remarkably similar to data recorded using organic solvents [6,14,24]. This result is important because the spectra recorded using organic solvents are from

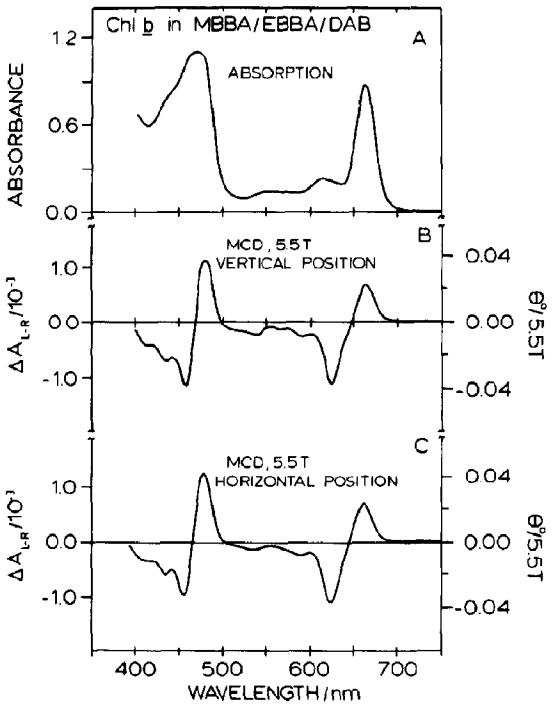

Fig. 2. Absorption and MCD spectra of Chl *b* dissolved in an MBBA + EBBA + DAB liquid crystal mixture, $c = 5 \times 10^{-3}$ mol l^{-1}. (A) Absorption spectrum. (B and C) MCD at 5.5 T, recorded with vertical (B) and horizontal (C) positions of the initial, zero-field liquid crystal orientation axis.

randomly distributed chromophores, whereas the liquid crystal system is highly anisotropic. In our previous study of these molecules at zero magnetic field [14], we found that the CD spectra were dominated by signals that could be associated with the helical alignment of the chlorophyll molecules that occurs as a result of the interaction of the liquid crystal solvent with the orienting SiO_x layer on the cell windows [14,23]. Because of this anisotropic arrangement of the chlorophyll molecules, the CD spectrum intensities were far greater than expected for randomly arranged chlorophyll molecules. The band intensities in the observed CD spectrum were highly dependent on the orientation of the sample cell itself [14].

We have used the rotation of the sample cell in the present study to check the extent of textural effects on the MCD spectrum. In both figs. 1B and 2B, the sample cell is arranged in a vertical position. The second of the two MCD spectra shown in figs. 1 and 2 was recorded with the sample rotated by 90° (a horizontal position). If the orientation of the liquid crystal molecules was not close to uniaxial along the direction of the light beam and magnetic field flux lines, very large changes would be seen in the relative amplitudes of the individual MCD bands in these two different orientations. Clearly, no major changes occurred.

However, the CD spectrum is very sensitive to the magnitude of the applied magnetic field [14]. When the magnetic field was reduced from 5.5 to 0.0 T, different effects were observed throughout the spectrum as the orientation of the liquid crystal solvent becomes influenced to a much greater extent by the SiO_x orienting layer on the cell windows; the spectrum now begins to show much greater change with respect to rotation of the cell.

It is possible to understand why the MCD spectrum at 5.5 T displays a signal that is so insensitive to the position of the cell by considering the magnetic field dependence of the liquid crystal solvent. At very high magnetic fields, the majority of the liquid crystal molecules align along the magnetic field flux lines; in the MCD experiment these are parallel to the light beam and perpendicular to the cell windows, so that we obtain the arrangement shown in fig. 3. Chlorophyll molecules are uniaxially oriented round the axis of the liquid crystal molecules, but not in other directions, as shown in fig. 3. The orientation of the porphyrin rings depends only on the solvation of the central Mg in the chlorophyll by the liquid crystal solvent molecules. The exact alignment differs for different chlorophylls but the axis of the liquid crystal is always located between

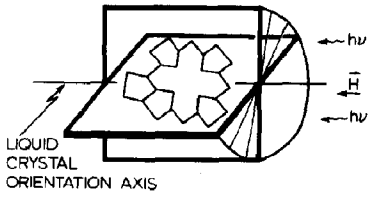

Fig. 3. A diagram showing the orientation of a single chlorophyll molecule and the liquid crystal solvent molecules in a strong magnetic field (H).

the B_x and Q_y transition moment directions [18,23,34]. The appearance of spectra that closely match those observed under isotropic conditions implies that the axis of the attachment of the liquid crystal molecule to the chlorophyll's porphyrin ring (both *a* and *b*) must lie close to 45° between the *x*- and *y*-directions, otherwise the shape of the envelope would be significantly perturbed. This result confirms suggestions made previously [14].

At lower magnetic field intensities, for example at half the field used normally, i.e., with $H = 2.75$ T, the liquid crystal and Chl *a* molecules located near the cell windows preserve their initial orientation parallel to the plane of the window, in contrast to the liquid crystal molecules in the bulk of the cell which are oriented parallel to the applied magnetic field (i.e., perpendicularly to the windows). This introduces a helical texture into the light path and CD intensity related to the liquid crystal is generated. At $H = 0.0$ T most of the liquid crystal molecules are oriented around an axis that has a direction parallel to the windows as a result of the SiO_x directing layer on the window surface. There is still some textural CD intensity that arises from the interaction of the bulk liquid crystal molecules with the surface layer.

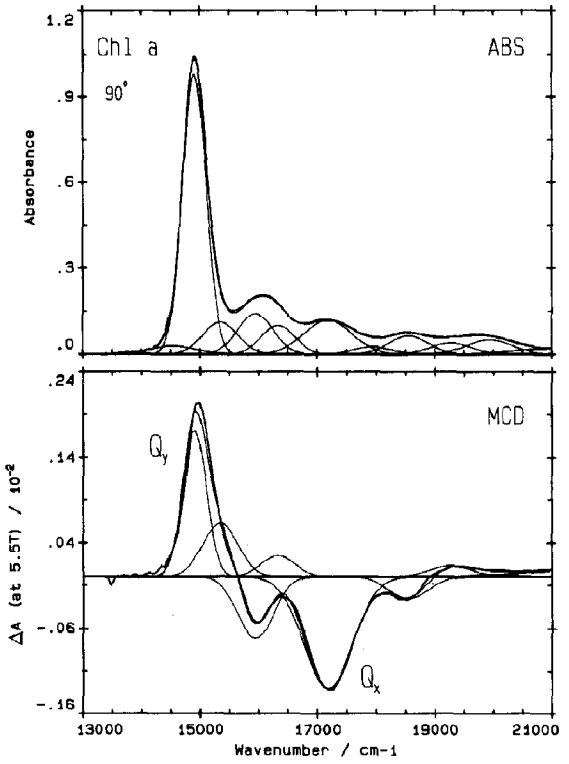

Fig. 4. Deconvolution of the absorption and MCD spectra of Chl *a* in the visible region.

3.4. Deconvolution of the absorption and MCD spectral data

The absorption and MCD spectra were fitted with Gaussian bands using the program SIMPFIT [44]. In these calculations, exactly the same parameters were used to fit pairs of associated components in the absorption and MCD spectra, i.e., the same number of components, the same band centres and half-widths [41]. Figs. 4–7 show the results of these calculations for the Q (figs. 4 and 6) and B (or Soret) band (figs. 5 and 7) regions. The individual parameters used are tabulated in tables 2 and 3. The fits shown here are relatively insensitive to small changes in starting parameters and, indeed, we obtained the same results for the spectra measured at both 0 and 90° orientations of the sample cells. Our approach of combining calculations for both absorption and MCD data is not the only way to deconvolute the spectra. We also fitted the absorption and MCD spectral data separately, unconstrained by the requirement that the parameters fit both sets of spectra. Under such conditions better fits were obtained, but the band centres and widths calculated by the program were not the same for both the absorption and MCD data. We believe that connection of the parameters determined from the absorption spectral data with those obtained from the MCD spectral data is essential if deconvolution calculations are to be carried out with data with as much overlap as is evident for the chlorophylls. This linking of the fitting significantly improves the reliability of the final results, leading to the only reasonable set of bands [41].

3.4.1. Deconvolution of the absorption and MCD data for Chl a

Two equivalent sets of spectra were used in the

Table 2

Band fitting parameters for Chl *a* at 90° orientation

The MCD B term was calculated as: $\langle \Delta A \rangle_0/(152.5)$. The absorption dipole was calculated as: $\langle A \rangle_0/(326.6)$. Data presented here were recorded for the same sample; the units of intensity have been left as ΔA and A; c_1 and H have not been factored in.

Band	ν (cm^{-1})	λ (nm)	$\Delta\nu$ (cm^{-1})	MCD B term (5.5 T)(10^{-7})	Absorption dipole (10^{-5})	B/D (10^{-3} T)
1	25526	392	971	0.47	6.00	+0.14
2	24630	406	966	−2.36	6.03	−0.71
3	23806	420	1081	−1.63	12.79	−0.23
4	23220	431	635	−1.94	2.77	−1.27
5	22762	439	819	7.04	11.22	+1.14
6	22032	454	731	0.42	0.71	+1.08
7	21097	474	1802	0.38	0.60	+1.17
8	19927	502	922	0.09	0.77	+0.28
9	19279	519	827	0.41	0.56	+1.33
10	18552	539	778	−0.73	0.89	−1.49
11	17944	557	770	0.02	0.37	+0.07
12	17179	582	959	−5.05	2.13	−4.32
13	16334	612	677	0.72	1.34	+0.98
14	15949	627	702	−2.20	2.03	−1.97
15	15351	651	703	2.03	1.64	+2.19
16	14911	671	472	3.81	10.12	+0.68
17	14529	688	906	−0.04	0.59	−0.11

Table 3

Band fitting parameters for Chl *b*

The MCD B term was calculated as: $\langle \Delta A \rangle_0/(152.5c)$. The absorption dipole was calculated as: $\langle A \rangle_0/(326.6)$. Data presented here were recorded for the same sample; the units of intensity have been left as ΔA and A; c_1 and H have not been factored in.

Band	ν (cm^{-1})	λ (nm)	$\Delta\nu$ (cm^{-1})	MCD B term (5.5 T)(10^{-7})	Absorption dipole (10^{-5})	B/D (10^{-3} T)
1	25611	390	1414	−0.875	3.16	−0.50
2	24219	413	1120	−1.32	2.67	−0.90
3	23605	424	850	−0.348	1.92	−0.34
4	23058	434	852	−0.957	1.96	−0.89
5	22298	449	1370	−2.60	9.26	−0.51
6	21575	464	879	−3.28	4.93	−1.23
7	20992	476	997	4.61	10.26	+0.82
8	19597	510	1056	−0.879	0.03	−59.20
9	18534	540	789	−0.702	0.14	−9.22
10	18102	552	871	−0.156	0.43	−0.66
11	17500	571	657	−0.139	0.27	−0.94
12	16851	593	1120	−1.28	0.58	−4.02
13	16423	609	579	−0.104	0.87	−0.22
14	16004	625	502	−2.30	0.40	−10.5
15	15539	644	730	−0.167	1.17	−0.26
16	15107	662	440	1.32	5.05	+0.67
17	14858	673	628	0.111	0.60	+0.34

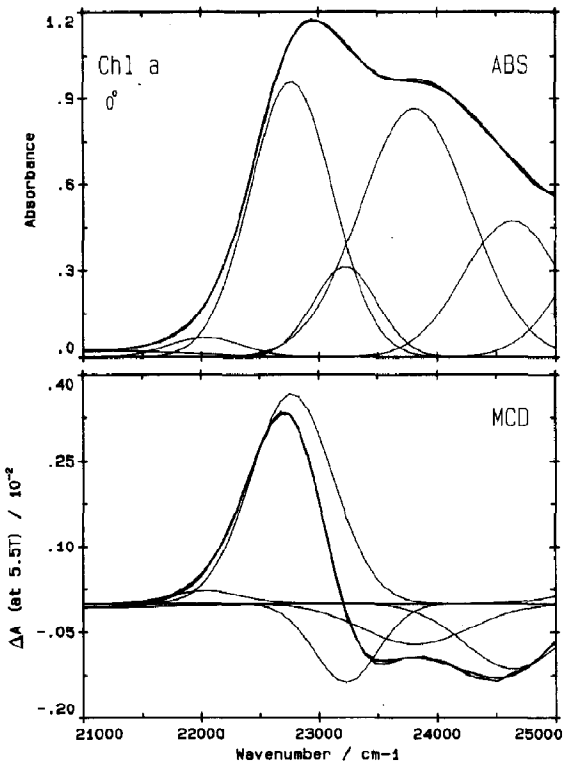

Fig. 5. Deconvolution of the absorption and MCD spectra of Chl *a* in the Soret band region.

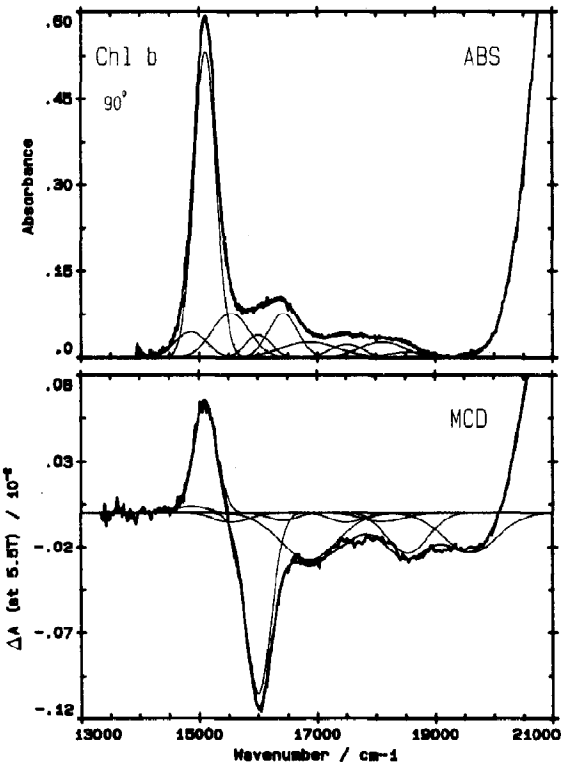

Fig. 6. Deconvolution of the absorption and MCD spectra of Chl *b* in the visible region.

deconvolution calculations for Chl *a*: (i) with the cell oriented at 0° and (ii) with the cell oriented at 90°. The sets of spectra recorded for both the 0 and 90° rotations gave the same results despite slight differences in the positions of the baselines. We report details here of only one of each of the sets of calculated data for each position. Fig. 4 shows the Q band region at 90° and fig. 5, the Soret band at 0°. The Q band region (taken here as the bands observed between 13 000 and 21 000 cm^{-1}) in each case was fitted with a total of ten components, of which eight make a prominent contribution to the absorption spectra, while only six contribute significant intensity to the MCD spectra. The Soret band region (above 21 000 cm^{-1}) (fig. 5) could be filled with five bands. This combination gave a very good fit to the MCD spectrum. Clearly, the blue end of the envelope, near 25 400 cm^{-1}, is a difficult region to fit, as liquid crystal absorption increases rapidly at higher energies, making the results more ambiguous above 25 000 cm^{-1}.

3.4.2. Deconvolution of the absorption and MCD data for Chl b

Two orientations were again considered, with the sample at 0 and at 90°. As the deconvolution calculations resulted in exactly the same band components, we discuss here only the results for the 90° orientation. The fitted spectral data are shown in figs. 6 and 7.

In the Q band region (fig. 6) the calculation required a single major band located at 15 107 cm^{-1}. A component at this energy filled both the absorption and the positive MCD envelopes. The components necessary to fill the rest of the MCD envelope controlled the deconvolution of the absorption envelope. Clearly, the negative MCD

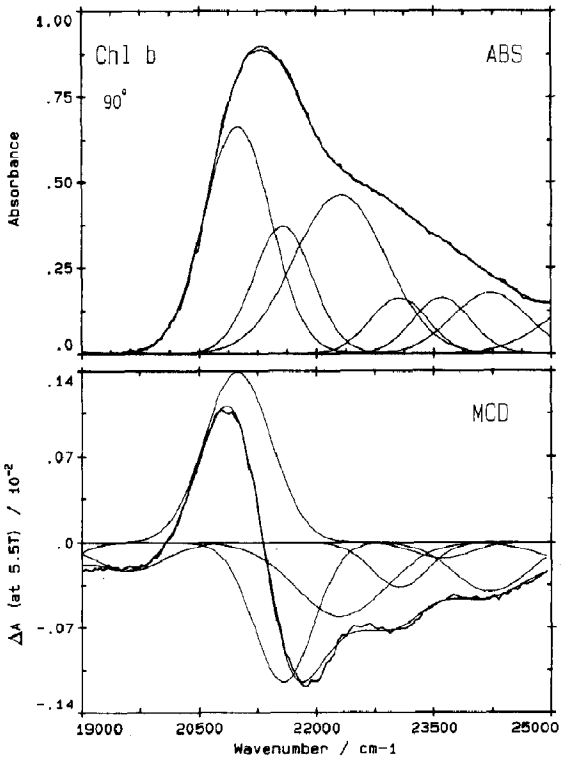

Fig. 7. Deconvolution of the absorption and MCD spectra of Chl *b* in the Soret band region.

band at 16004 cm^{-1} is associated with a very weak absorption band. The lack of resolution between 16004 and 19500 cm^{-1} means that it is difficult to determine accurately the energy of the bands responsible for both absorption and MCD signal intensities. In the Soret region between 19000 and 25000 cm^{-1}, we find that six components are required (fig. 7). The MCD spectrum quite closely controlled the possible combinations that would fill the absorption spectrum. Clearly, the major MCD intensity is associated with the major absorption bands.

3.5. Interpretation of the transitions identified by fitting the chlorophyll absorption and MCD spectra

Several theoretical calculations have been published that describe the number and polarization of bands in the absorption spectra of chlorophylls and models of chlorophylls [1,11,12,15,26]. Several influences are well known to perturb the spectrum of chlorophyll [1,11,12,18,22,24,29–31], including the arrangement of peripheral substituents, solvent dielectric constant, coordination of the central Mg (primarily, whether it is penta- or hexacoordinated), extent of aggregation and whether the chlorophyll is in solution or in the solid state. The major effect of these perturbations can be (i) an alteration in the order of the *x*- and *y*-components of the Q and B bands, (ii) a change in the relative intensities of each of the components, and (iii) a shift in energy of the whole spectrum. In the liquid crystal matrix, we anticipated that perturbations from the method of solvation between the liquid crystal solvent and chlorophyll molecules, and the metal coordination number, would result in greatest modification of the spectrum recorded compared with those recorded under isotropic conditions. It is clear from our data that, at 5.5 T, these effects are not pronounced and that the MCD spectral data closely reproduce the spectra recorded isotropically, with the exception of a red shift, when compared with spectral data recorded for Chl *a* and *b* dissolved in diethyl ether [1,6].

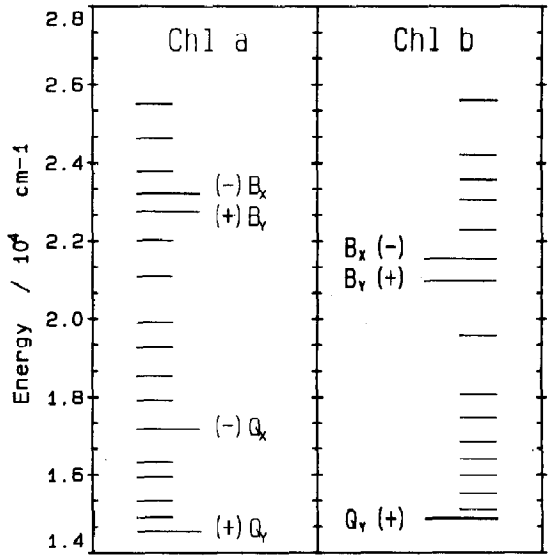

Fig. 8. Energy level diagram showing spectral components for Chl *a* and *b* in the liquid crystal matrices obtained by deconvolution of the absorption and MCD spectra.

Fig. 8 collects together the results of the deconvolution calculations carried out in this study in the form of an energy level diagram. We have identified the energies of the Q and B components in both Chl *a* and *b* by reference to the location of high values for B/D from the MCD spectra [1]. There are many more components listed in tables 2 and 3, and plotted in fig. 8, than are commonly reported [15]. Because we do not find any suggestion of aggregation, we do not consider that these extra components arise from exciton coupling. Rather, we suggest that the other components required to fill both the absorption and MCD envelopes arise from vibronic coupling to the pure electronic states [39].

3.5.1. Chl a

The assignment of the Q band of Chl *a* in the liquid crystal matrix is straightforward. $Q_y(0\text{-}0)$ is at 671 nm [1] and is clearly associated with the positive *B* term. Although the absorption spectrum is not so distinct, $Q_x(0\text{-}0)$ can be found from the MCD spectrum at 582 nm. The deconvolution calculation shows that several other absorption bands are associated with MCD components. Because of the constraint of fitting both absorption and MCD spectra, we locate three bands between Q_y and Q_x, at 651, 627 and 612 nm. Similarly, following Q_x, we find three bands, at 557, 539 and 518 nm.

The assignment of each of the bands in the Soret region has long been a problem because the splitting between the *x*- and *y*-components is not as pronounced as for the Q band [1]. The overlap of these two, oppositely polarized transitions is readily apparent in the MCD spectrum. Because the two oppositely signed *B* terms are so close together, a derivative-shaped envelope is seen, thus isolated $B_y(0\text{-}0)$ and $B_x(0\text{-}0)$ bands are not observed. It has also been predicted from theoretical calculations [1,26] that the Soret band in chlorophylls includes several intense transitions, with transition moments aligned along various directions in the plane of the porphyrin plane [1,26]. The deconvolution of both absorption and MCD spectra allows fairly precise determination of the energies of these two transitions, with B_y at 22762 cm^{-1} (439 nm) and B_x at 23220 cm^{-1} (431 nm).

3.5.2. Chl b

Unlike the situation for Chl *a*, finding both components in the Q band region of Chl *b* is difficult. While $Q_y(0\text{-}0)$ clearly lies at 662 nm, it is unclear where $Q_x(0\text{-}0)$ is located from the absorption spectrum alone. It is usual for the MCD spectrum to provide the extra assignment criterion necessary to locate the split pairs of Q bands in the spectra of chlorins [1,3,10]. For Chl *b* we have to choose between the strong negative MCD band at about 625 nm (band 14, table 3) and a band identified from polarized emission spectra near 540 nm. In both cases the spectral information is not conclusive. While the MCD band at 625 nm reflects the normal pattern of alternating signs [1,3,10], the absorption spectrum is very weak. This intense MCD transition at 625 nm exhibits an unusually large and negative B/D value as the band is observed under both anisotropic and isotropic conditions and is not dependent on the attachment of the chlorophyll molecule to the liquid crystal solvent or on the extent of aggregation. If this band is not $Q_x(0\text{-}0)$ then there must be unusually efficient vibronic coupling in the Chl *b* that results in such a strongly *x*-polarized transition. Neither the absorption nor the MCD spectrum can really be used to support the 540 nm position, although for more symmetric chlorins the $Q_x(0\text{-}0)$ band is found to be blue shifted compared with Chl *a* [3]. The MCD spectrum allows for the unambiguous identification of B_y at 20992 cm^{-1} (476 nm), with B_x located at 21575 cm^{-1} (464 nm). The blue side of the Soret band is more difficult to resolve because several bands of significant intensity in both the absorption and MCD spectra overlap.

3.6. Comparison between the spectral analyses of Chl a and Chl b in the liquid crystal matrix and data recorded isotropically

No other deconvolution data are available for chlorophylls dissolved in a matrix like a liquid crystal. Deconvolution calculations have been reported for parts of the absorption spectra of Chl *a* in organic solvents only [15,22–24].

Absorption and MCD spectra for several different chlorophylls dissolved in a variety of

solvents have been reported by Weiss [1], Belkov and Losev [24], Schreiner et al. [6], Schooley et al. [5] and Keegan et al. [12]. The most extensive deconvolution calculations to date have been reported by Shipman and co-workers [22]. In the work of Shipman et al. [22], the deconvolution of the visible region of the absorption spectra of Chl *a* was described. The Mg in the Chl *a* was either penta- or hexacoordinated and, in addition, calculations were carried out for spectra recorded for a progressive increase in the extent of oligomerization [22]. In the absence of MCD data that could be used to constrain the band centre and bandwidth parameters, Shipman et al. [22] simply used a five-band fit between 14000 and 20000 cm^{-1}. The first three components were centred on band maxima clearly observed on the blue side of the main transition in the spectral envelopes, the fourth and fifth being fitted together under the Q_y band near 670 nm (14900 cm^{-1}).

In each of our fits, we have constrained the calculation rather severely to fit both absorption and MCD spectral envelopes with only changes to intensities being allowed. The well-resolved and narrow bandwidths observed for Q_y in both Chl *a* and *b* strongly support the results of the resonance Raman experiments [49] that Chl *a* in EBBA + MBBA is monomeric. These data also conform to those expected for monomeric species in isotropic solutions, for example, for Chl *a* in diethyl ether [22]. Clearly, as the Mg in Chl *a* is pentacoordinated [49], there must be a large red shift imposed by the liquid crystal matrix, such that the Q_y and Q_x bands are located some 10 nm from the values measured in ether [1,25]. Red shifts are also observed for Chl *a* in this films and monolayers [4,19–21]. Significantly, our deconvolution calculations for Chl *a* in the liquid crystal matrix fill the Q_y band almost entirely with a single component, the next band lying approx. 19 nm to the blue with considerably less intensity. This compares with the two overlapping bands used by Shipman et al. [22] to fill the Q_y envelope under isotropic conditions. The absence of oligomerization implies that all components obtained by deconvolution analysis, with the exception of the (0-0) bands, must be vibronic in nature rather than the results of exciton splitting.

Deconvolution of the Soret absorption band of Chl *a* in pyridine has been reported only by Journeaux and Viovy [23]. Despite the onset of absorption due to the liquid crystal solvent above 25000 cm^{-1}, the results from our calculations resemble quite closely the bands obtained in pyridine [23]. In particular, we reproduce the location of the first three bands very well.

The situation for Chl *b* is not as clear as for Chl *a*. Resonance Raman [50] data indicate that complexation with the liquid crystal molecules is through the Mg atom and the peripheral aldehyde on the chlorophyll to either another chlorophyll to form a dimer, or to a single liquid crystal molecule. In both cases it is expected that the chlorophyll will be pentacoordinated. The spectra of Chl *b* in the liquid crystal matrix are clearly of monomeric species, with a Q band spectrum similar to that of Chl *b* in diethyl ether [25], with the exception of the 20 nm red shift in the liquid crystal solvent.

Comparison between the data for Chl *b* (table 3) and those for Chl *a* (table 2) indicates that the *B/D* values for Chl *b* bands are, in most cases, higher than those for Chl *a*. The sequence of signs for the MCD bands for both pigments is the same, but the *B/D* amplitudes are quite different. Schreiner et al. [6] concluded that the MCD spectra of chlorophylls are very sensitive to the change from the Chl *a* to *b* substituents (the difference between Chl *a* and *b* is in substitution at position 3 on ring II). These authors [6] found a different sequence for the signs of the *B* terms for Chl *a* (+ − −), from long to short wavelengths, from that which they observed for Chl *b* (+ − +). A similar effect has been examined by Djerassi and co-workers [11,12] for chlorins. Our MCD data for Chl *a* and *b* are slightly different from those reported by Schreiner et al. [6] and our fits do not give the same bands as deduced by those authors. However, the MCD intensities are so low on the blue side of the Q band envelope that the position of the baseline can change some of the signs of the weak bands.

It is clear that the *B/D* values for Chl *b* are different from those of Chl *a* in the Soret region. It is difficult to comment further on how general this phenomenon is because so few spectral data

are available for Chl *b* and no quantitative results have been reported previously for MCD data for Chl *b*.

Because the sign of the *B* terms that lie under the Q band components is so sensitive to the peripheral substituents of the porphyrin ring [6,11,12], we can suggest, as a consequence of the appearance of a quite normal, 'isotropic-like' MCD spectrum for both Chl *a* and *b* in the liquid crystal matrix, that there is indeed little perturbation of the π ring and its peripheral groups by the solvating LC molecules [50].

4. Conclusions

(1) The MCD spectra of Chl *a* and Chl *b* recorded at high magnetic fields have similar band envelopes in the liquid crystal solvents to those in isotropic solvents.

(2) A quantitative band analysis has been carried out based on both the absorption and MCD spectra in the Q and B (Soret) band regions. This is the first time that such an extensive analysis has been reported for these two important chlorophyll derivatives.

(3) The liquid crystal: Chl *a* interaction appears to be so strong that aggregates between chlorophylls do not form until much higher concentrations are reached than those used in this study, i.e., greater than 10^{-3} mol l^{-1}. Therefore, Chl *a* is monomeric in the sense that it interacts only with the liquid crystal molecules. This means that in the terminology used for chlorophylls in vivo, we have only one Chl *a* form present; this has a Q band maximum at 671 nm. This band is found to be *y*-polarized as expected, and is assigned as the $Q_y(0\text{-}0)$ transition.

(4) Chlorophyll molecules dissolved in the liquid crystal matrix are good models for the antenna systems proposed by Beddard and Porter [50]. These authors suggest that although the chlorophylls in the antenna assemblies are monomeric the separation of the pigments results from strong interactions with other molecules, a situation very similar to that found for Chl *a* and *b* dissolved in the liquid crystal matrix.

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