ABSORPTION SPECTROSCOPY OF CHLOROPHYLL IN BIMOLECULAR LIPID MEMBRANES

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SUMMARY

A technique has been developed for measuring absorption spectra of pigments incorporated into lipid membranes. Spectra have been obtained for chlorophylls a and b in lecithin membranes and used to estimate the composition of the membrane. Polarised spectra show that the ratio of the blue and red absorption maxima is dependent on the angle between the plane of the bilayer and the electric vector of the incident light. This indicates that the porphyrin rings of chlorophyll are at least partially oriented and that the transition moments of these two absorption bands are not parallel.

The knowledge that the pigments in the lamellae of chloroplasts and retinal rods exhibit a marked degree of organisation encouraged considerable research into the solid state properties of chlorophyll, carotenoids and related molecules (1). The extrapolation of the results of these studies to the *in vivo* situation has, however, always been speculative. It has previously been suggested

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that further insight might be gained by studying the properties of pigments incorporated into artificial lipid membranes (1,2) and some progress has already been made in this direction (3-6).

One of the principal attractions of the model system is that it offers the possibility of isolating and studying particular processes in a membrane of well defined structure and composition. In the case of pigment-lipid membranes, spectroscopic techniques should in principal be of assistance in characterising the system. However, difficulties arise in practice due to the extreme thinness of the lipid bilayer ($\sim$ 100Å); indeed Van and Tien (4) have taken a pessimistic view of the possibility of measuring optical absorption with presently available spectrophotometers.

Calculation of the maximum absorption to be expected from a lipid bilayer containing 10% chlorophyll indicates a value in the order of 1% (see below). Such a value is detectable but too low to obtain any detailed spectroscopic information. We have therefore constructed a cell which enables a number of bilayers to be placed in the path of the light beam (Figure 1). The cell is made from perspex and equipped with optical quartz windows at either end. The bilayers are formed by the brush technique across 4mm circular apertures in teflon plates which slide into the cell. The plates are tilted at 45° to the incident light beam for ease of visual observation. The whole cell fits into the sample space of a Cary 14 spectrophotometer.

Chlorophylls a and b were extracted from pea leaves and purified on preparative thin layer chromatography plates

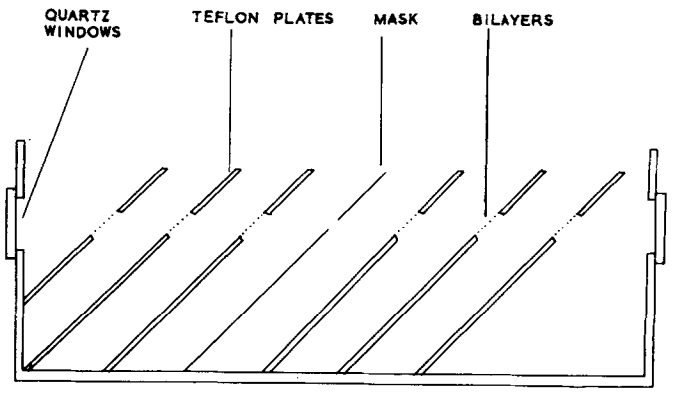

Figure 1. Schematic Diagram of Absorption Cell.

using a procedure derived from that of Hager and Bertenrath (7). Lecithin was extracted from egg yolks and purified on alumina and silicic acid columns as described by Dawson (8). Membranes were formed from solutions of 1% lecithin in n - decane (Koch Light Puriss) to which various amounts of chlorophyll had been added. The aqueous phase consisted of 0.1M KCl.

The procedure for measuring absorption spectra was as follows. The series of apertures was first carefully aligned in the spectrophotometer and the cell filled with the KCl solution. A base line was then run over the spectral range to be examined. Membranes were formed across the apertures and allowed to thin to the black or bilayer state. The absorption spectrum was then measured, the bilayers broken and the base line rechecked. In some experiments the bilayers were broken sequentially and further spectra run at each stage.

RESULTS AND DISCUSSION

Absorption spectra for chlorophylls a and b in the

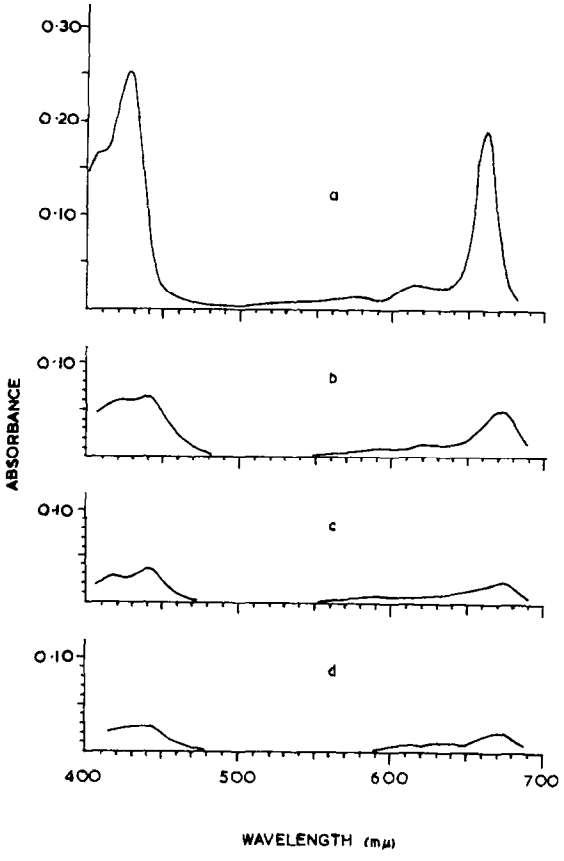

Figure 2. Absorption Spectra for Chlorophyll a.

(a) Solution spectrum in diethyl ether.
(b) Unpolarised spectrum of 6 bilayers.
(c) Horizontally polarised spectrum of 4 bilayers.
(d) Vertically polarised spectrum of 4 bilayers.
Ratio of chlorophyll*a*-lecithin in membrane forming solution is 1.2:1 in (b) and 0.75:1 in (c) and (d)

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lecithin bilayer are shown in figures (2) and (3). Experiments with various masking arrangements established that the observed absorptions were due to the bilayer and not the surrounding Gibbs ring. Compared with the spectra of bulk solutions in ether (or decane) the bands show a pronounced shift to the red. The spectroscopic data are collected together in Table 1.

The magnitude of the red shifts is comparable to that

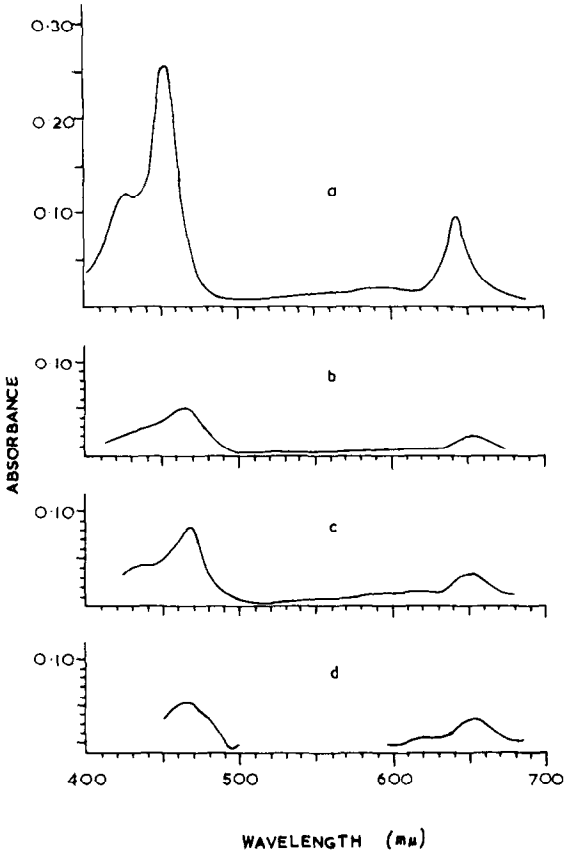

Figure 3. Absorption Spectra for Chlorophyll b

(a) Solution spectrum in diethyl ether.
(b) Unpolarised spectrum of 5 bilayers.
(c) Horizontally polarised spectrum of 5 bilayers.
(d) Vertically polarised spectrum of 5 bilayers.
Ratio of chlorophyll b-lecithin in membrane forming solution is 1:1 in all cases.

observed in lipid-chlorophyll dispersions (9) and in chlorophyll monolayers (10-12). Bellamy, Gaines and Tweet (12) found that the positions of the bands were independent of the surface pressure over the range 1-14 dyne/cm and hence deduced that the shift was most probably a solvent effect rather than a consequence of interaction between chlorophyll molecules. The present studies indicate that the positions of maximum absorption are

independent of chlorophyll concentration over the range studied and hence support this interpretation.

The concentration of pigment in the bilayer may be calculated as follows. If y is the number of pigment molecules/cm^2 in the bilayer the molar concentration c is given by $c = My/dN$, where d is the bilayer thickness, M the molecular weight of the pigment and N is Avagadro's number. Then the absorbance A is given by

$$A = \varepsilon cd = \varepsilon yM/N \qquad (1)$$

where ε is the molar extinction coefficient.

Inserting the measured values of absorbance into equation (1) and using values of ε obtained in ether (13) we calculate a value of $y = 4.2 \times 10^{13}$ for bilayers formed from lecithin-chlorophyll b 1 : 1 and a slightly lower value of $y = 3.6 \times 10^{13}$ for lecithin-chlorophyll a 1:1. If we assume that to a first approximation the surface area of lecithin is unaffected by the introduction of chlorophyll, we may also deduce that the number of lecithin molecules/cm^2 of bilayer is 3×10^{14} (taking 66Å^2 as the area/mole (14)). Thus the mole ratio of lecithin-chlorophyll in the bilayer in the above instances is 7.2:1 for chlorophyll b and 8.3:1 for chlorophyll a. As would be expected these values vary with the proportion of chlorophyll in the membrane forming solution. We have formed bilayers from solutions of lecithin-chlorophyll in the range 5:1 to 1:2. Over this range the proportion of chlorophyll in the bilayer is consistently about an order of magnitude smaller than that in the initial solution. The above values must be regarded as estimates since the value of ε in the bilayer may be different from that measured in solution.

TABLE 1

Spectroscopic Data for Chlorophylls *a* and *b* in Lecithin

Bilayers

	Wavelength of Absorption Maxima (mμ)			Intensity Ratio Blue Peak/Red Peak		
	Blue Satellite	Blue Peak	Red Peak	Unpolarised	Horizontal Polarisation	Vertical Polarisation
Chl *a*	420	440	672	1.2	1.7	1.3
Chl *b*		465	651	2.2	2.8	1.4

Information about the orientation of chlorophyll in the bilayer may be obtained from polarised absorption spectra. In the case of both chlorophylls *a* and *b* the ratio of theblue and red peak intensities is increased when the incident light is polarised perpendicular to the plane of incidence (i.e. horizontally, see Table 1). From this result we may conclude that there is some degree of orientation of the porphyrin rings of chlorophyll in the bilayer and that the transition moments of the red and blue bands must lie in different directions. Further studies of polarisation effects together with reflectance measurements are in progress in an attempt to gain some further information about the orientation of chlorophyll in the bilayer and the direction of the transition moments of the two bands.

In conclusion, the present experiments demonstrate the feasibility of using polarised absorption spectroscopy to obtain details of composition and structure of pigment-lipid bilayers. The sensitivity is such that in favourable instances (i.e. where the absorption to be measured has comparable intensity to that of chlorophyll), it is possible to obtain useful information

with as little as 2% pigment in the bilayer. With bilayers of good stability this sensitivity could conceivably be improved still further by the use of computer averaging techniques. Thus it is anticipated that the method may be readily extended to the study of other molecules, such as vitamin A derivatives, carotenoids, cytochromes and polyene antibiotics, which have suitable u.v. or visible absorptions.

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