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CHLOROPHYLL *b* CONTAINING LIPOSOMES: EFFECT OF THERMAL TRANSITIONS ON CATALYTIC AND SPECTRAL PROPERTIES

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SUMMARY

1. Chlorophylls *a* and *b* were incorporated into saturated and unsaturated phosphatidylcholine liposomes. The red absorption bands of the two chlorophylls were at 671 nm and 651 nm, respectively, both in liposomes containing egg phosphatidylcholine and in liposomes containing saturated lecithins above their transition temperatures.

2. In dipalmitoylphosphatidylcholine liposomes the absorption bands of chlorophyll *b* show a red shift on cooling below the transition temperature. Chlorophyll *b* broadens and depresses the transition temperature region of hydrated dipalmitoylphosphatidylcholine.

3. Chlorophyll *b* containing liposomes catalyse the reduction of cytochrome *c* by trimethylhydroquinone in a light-sensitive reaction; the catalysis by egg lecithin liposomes has a rate about 60 % that of Triton-dispersed chlorophyll and shows no sharp temperature effects, but the catalysis by dipalmitoyllecithin liposomes is only seen above their transition temperature.

4. Chlorophyll *b* containing dimyristoyllecithin liposomes show a smaller spectral shift and a less marked increase in catalytic activity on passing through their transition temperature.

5. Chlorophyll *a* containing saturated liposomes show smaller spectral and catalytic responses to temperature than do the chlorophyll *b* containing liposomes.

INTRODUCTION

A number of phenomena have recently been shown to be linked to the thermal transitions in phospholipid membranes, including changes in permeability [1, 2], in enzyme activity [3, 4], and in the fluorescence of appropriate "probes" [5]. Similarly the electron transport mechanisms of mitochondria are blocked at low tem-

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perature [6], although this may be due to a continuously high activation energy rather than to identifiable transitions at one or more specific temperatures. On the other hand the photosynthetic oxidation of *c*-type cytochromes in bacterial chromatophores is remarkably insensitive to lowering the temperature [7], appreciable reactivity remaining at 4 °C (and, by extrapolation, at 0 °K).

It has been previously reported that chlorophyll *a* dissolved in aqueous phospholipid suspensions (liposomes) has properties such that: (a), its spectrum is shifted from 661 nm (diethyl ether) or 663 nm (acetone) to 670–673 nm in phospholipid [8–10]; (b), it is capable of photocatalysed reduction of added cytochrome *c* in a hydrogen donor independent reaction [8]; and (c), it shows an optical transient on flash photolysis with a half-life of about 0.5 ms, but only in unsaturated liposomes [9].

The behaviour of chlorophyll in such systems is also interesting because: (i), a "natural" photo-activated electron transfer reaction might be capable of being used to create a transmembrane potential or pH gradient (cf. Deamer et al. [11]) and (ii), the changes occurring above and below the transition temperature in chlorophyll-containing vesicles could throw light on the question of chilling sensitivity and resistance [12].

We have, therefore, examined the effects of temperature on the spectrum and catalytic activity of chlorophyll in liposomal suspensions. In the case of chlorophyll *b*, we have been able to demonstrate a correlated change in these two properties on passing through the transition temperature region of saturated phospholipids★.

MATERIALS AND METHODS

Chlorophylls a and b

These were prepared from fresh pea shoots (Laxton's superb). Shoots (80 % water) were homogenized (Ultra–Turrax) in acetone to give a final acetone concentration of 80 %. The acetone was filtered through celite and extracted with light petroleum (60–80 °C) (100 ml/500 ml acetone extract). The light petroleum fraction was washed several times with water and then with methanol (A.R.) until the washings became green instead of yellow in colour. The light petroleum layer was then washed again with water containing a trace of $MgSO_4$ and NaCl (to break emulsions) and the organic phase evaporated to dryness under a stream of O_2-free N_2.

The pigments were separated on powdered sugar columns, as described in Smith and Benitez [14]. A column containing 300 g powdered sucrose was developed with light petroleum (60–80 °C)+0.5 % *n*-propanol until the main chlorophyll *a* fraction was eluted; the main chlorophyll *b* fraction was then eluted with light petroleum (60–80 °C)+0.5 % *n*-propanol+4 % ethanol. These fractions were evaporated to near dryness in vacuo. The products contained > 60 % chlorophyll by weight and spectroscopic analysis. Other pigments (carotenoids etc.) were not detectable, and cross-contamination of the two chlorophyll species was negligible.

The residual material was probably mostly water, but may have included some

★ A preliminary version of this paper was communicated to the 8th FEBS Meeting, August 1972, in Amsterdam [13]. This abstract erroneously describes a "blue shift" on "cooling" chlorophyll-containing liposomes of saturated phospholipids. This should have read either as a "blue shift on warming" or "red shift on cooling" the chlorophyll *b* containing dipalmitoylphosphatidylcholine liposomes.

colourless neutral lipid with chromatographic behaviour resembling that of the chlorophyll. Repeated chromatography was not attempted because of the tendency shown by chlorophylls to "allomerize" and to form phaeophytins. Chlorophyll *a*, and to a much lesser extent chlorophyll *b*, did tend to form phaeophytin on standing. The possibility that small amounts of impurities are contributing to the phase transition effects (below) must be borne in mind.

Liposomes

Lipids plus chlorophyll dissolved in chloroform were mixed in 30-ml stoppered glass tubes with venting side-arms. Usually 50–250 μl of lipid solution (5–50 mM) plus 50–125 μl chlorophyll ($\approx$ 1 mg/ml) were used, together with "control" mixtures containing no chlorophyll. To ensure even mixing, about 1 ml chloroform was then added to each tube. The tubes were evaporated to dryness, swirling the liquid continuously to produce an even film occupying at least 1 cm height from the base of the tube. 1–5 ml 20 mM Na_2HPO_4/KH_2PO_4 buffer (pH 6.8) were added to swell the liposomes, using a Vortex mixer. With saturated lipids, the tubes were warmed above the transition temperatures and a few ballotini added to ensure complete removal of the lipid film. The resulting chlorophyll-containing liposomes were kept dark and used within 1–2 h of preparation.

Spectrophotometry

A Perkin–Elmer 402 spectrophotometer was used for all experiments except that of Fig. 1 (standard spectra obtained in a Unicam SP-800). Difference spectra (Figs 2 and 7) were measured with diluted liposome suspensions in 3-ml standard 1-cm cuvettes in a thermostatically-controlled cell holder (sample plus reference). Reaction rates (Figs 5, 6 and 8) were followed using an individually thermostatted cuvette (water-jacketed semi-micro type) with path length 1 cm and capacity 1.5 ml. Actinic red light was supplied to the sample cuvette by a 12-V, 50-W, QI lamp mounted on the front of the cover of the cell compartment. The light passed a focussing lens and a Wratten 23A (red) filter through a 1.0-cm diameter aperture 16 cm from the cuvette, and passed down a matt black tube at 90 ° to the analysing beam. The QI lamp was cooled by a small fan. The water-jacketed portion of the front face of the cuvette was blackened, and a corresponding blackening was carried out with the (standard) reference cuvette. The photomultipliers were shielded from the red actinic light by a pair of blue filters (Wratten No. 47B) transmitting light between 380 and 490 nm (max. 435 nm). Turning on the actinic light had no effect on the cuvette temperature and produced only a small increase in "noise" on the recorder without switching transients.

The matched filters thus necessitated measurement of cytochrome *c* reduction at 420 nm (instead of the usual 550 nm).

Differential scanning calorimetry (Fig. 3)

A Perkin–Elmer DSC-1B instrument was used (experiment carried out by J. Hoyland).

Materials

Egg phosphatidylcholine and phosphatidic acid were prepared by Nigel Miller and were > 99 % pure by chromatographic analysis. Dipalmitoyl- and di-

myristoylphosphatidylcholine were obtained from Sigma, London, Ltd, and found to contain > 98 % of the indicated fatty acid by gas–liquid chromatography analysis. Cytochrome *c* was Sigma horse heart Type III. Other reagents were from standard commercial sources.

Trimethylhydroquinone was prepared from a stock solution (≈ 100 mM) kept at 6 °C. Freshly prepared solutions (from the solid quinol) were sometimes found to show rather sluggish initial responses, probably due to low levels of the quinone (cf. Fig. 4), which could be overcome by the addition of a small quantity of oxidized solution. The level of trimethylhydroquinone added was adjusted to give a maximum ratio between the rates of the light and dark reactions, at any given concentration of chlorophyll liposomes. Under such conditions the rate of the light reaction was proportional to the effective chlorophyll concentration.

RESULTS

Chlorophylls *a* and *b* were mixed with lipid and resuspended in dilute buffer solutions to form chlorophyll-containing liposomes, as described above. In both

TABLE I

POSITION OF α-PEAKS OF CHLOROPHYLLS *a* AND *b* IN SOLVENTS AND LIPOSOMES

Conditions of chlorophyll incorporation into liposomes as described in Materials and Methods. Concentrations from 2.5–12 μM in the various solvents at 28 °C. Chlorophyll: phospholipid ratio in liposomes approx. 1 : 25.

Solvent	Peak (nm)	Liposomes	Peak (nm)
Chlorophyll *a* α-peak***			
Diethyl ether	661	Egg phosphatidylcholine (25 °C)	670–671
Acetone	663		
Chloroform	665	Dipalmitoylphosphatidylcholine (50 °C)	671–672
Benzene	666	Dipalmitoylphosphatidylcholine (25 °C)	671–673*
Aqueous Triton X-100 (1 %)	668		
Chlorophyll *b* α-peak			
Light petroleum	639	Egg phosphatidylcholine (20–50 °C)	651–652
Diethyl ether	640		
Acetone	644	Dipalmitoylphosphatidylcholine (50 °C)	651
Benzene	645	Dipalmitoylphosphatidylcholine (20 °C)	654**
Chloroform	646	Dimyristoylphosphatidylcholine (35 °C)	650
Aqueous Triton X-100 (0.5–1.5 %)	645–649	Dimyristoylphosphatidylcholine (20 °C)	652**
Aqueous acetone (50 %)	650		

* No distinct shift on cooling (see text).

** Red shift on cooling (see text).

*** In other organic solvents the α-peak may occur at longer wavelengths (e.g. 671 nm in pyridine, 674 nm in aniline) [8].

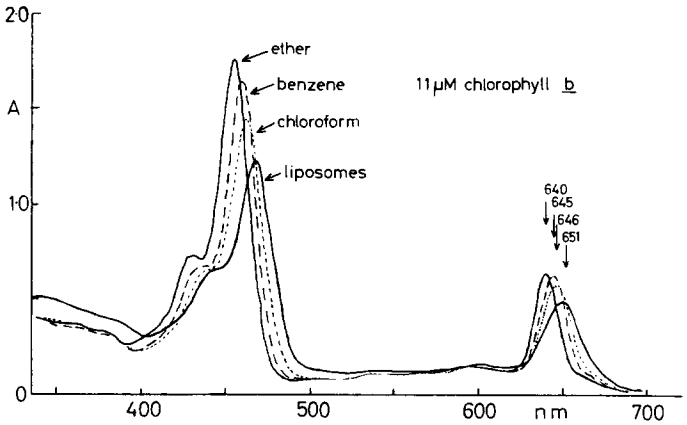

Fig. 1. Absolute spectra of chlorophyll *b* in organic solvents and liposomes. 11 μM chlorophyll *b*, 28 °C. ———, diethyl ether; – – –, benzene; - - -, chloroform. ———, liposomes (chlorophyll–liposomes vs liposomes alone) containing 0.5 μmole egg phosphatidylcholine 0.02 μmole egg phosphatidic acid, + 0.011 μmole chlorophyll *b*/ml in 20 mM phosphate buffer (pH 6.8).

cases the α-peaks were found at longer wavelengths than in the usual solvents (Table I), although the positions are similar to those of chlorophylls dispersed in 1 % Triton X-100 solution and in 50 % aqueous acetone. With the saturated phospholipids, the position of the α-peak was a function of temperature, but with egg lecithin liposomes temperature variation had little effect on the spectrum (Table I).

Chlorophyll *b* was found to exhibit more marked temperature-dependent

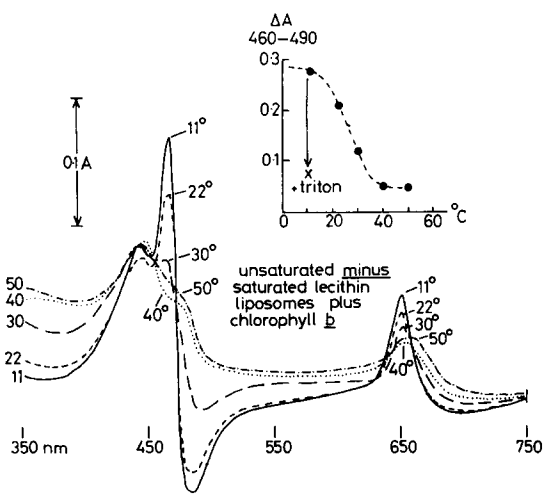

Fig. 2. Difference spectra of chlorophyll *b* in unsaturated and saturated liposomes. 11 μM chlorophyll *b* in egg phosphatidylcholine liposomes (sample) minus chlorophyll *b* in dipalmitoylphosphatidylcholine liposomes (reference cuvette), phosphate buffer 20 mM (pH 6.8). Inset: Effect of adding approx. 0.1 % Triton X-100 on the difference at 460–490 nm. ———, 11 °C; - - -, 22 °C; – – –, 30 °C; · · ·, 40 °C; – · – · – · –, 50 °C. Liposomes contained 0.5 μmole lecithin (egg or dipalmitoyl), 0.02 μmole phosphatidic acid (egg or dipalmitoyl) + 0.011 μmole chlorophyll *b*/ml.

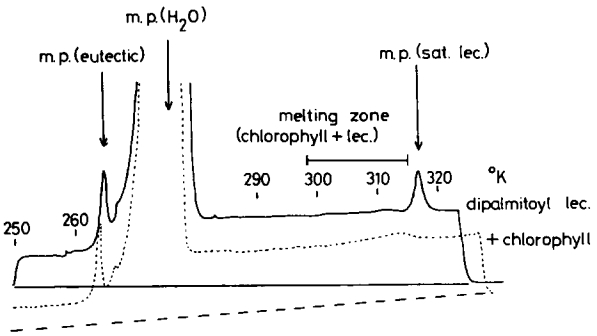

Fig. 3. Differential scanning calorimetry of dipalmitoyllecithin liposomes in presence and absence of chlorophyll *b*. Dipalmitoyllecithin liposomes (———) or similar liposomes containing 3 % chlorophyll *b* (- - -) dispersed in a minimal amount of phosphate buffer (pH 6.8).

spectral shifts than chlorophyll *a* and further studies were, therefore, made with the former species; its relative stability against Mg^{2+} loss (phaeophytin formation) also makes it the favoured material for investigation. Fig. 1 shows the absolute spectra of chlorophyll *b* in ether, benzene, chloroform, and liposomes (using pigment-free liposomes in the reference cuvette). In addition to a red shift of both blue and red peaks, the liposomal chlorophyll *b* shows a decrease in apparent extinction coefficient. A similar red shift and "bathochromicity" are seen on the addition of water to acetone solutions of chlorophyll *b*, possibly reflecting pigment aggregation.

When the spectrum of chlorophyll *b* in egg lecithin liposomes is compared with that in dipalmitoyllecithin liposomes, a temperature-dependent peak shift is seen in the latter system (Table I). Fig. 2 records the difference spectra for chlorophyll *b* in egg phosphatidylcholine liposomes (sample) minus the same quantity of chlorophyll *b* in dipalmitoylphosphatidylcholine liposomes (reference). Below the transition temperature for the latter liposomes, the chlorophyll shows a red shift in both the blue and red regions; above the transition temperature only a slight bathochromicity is seen in the reference cuvette. The inset shows that the change, as measured by the 460–490 nm difference, is about half-maximal at 27 °C, and maximal at 10 °C (but abolished by addition of Triton X-100 to both cuvettes). Normally the transition tem-

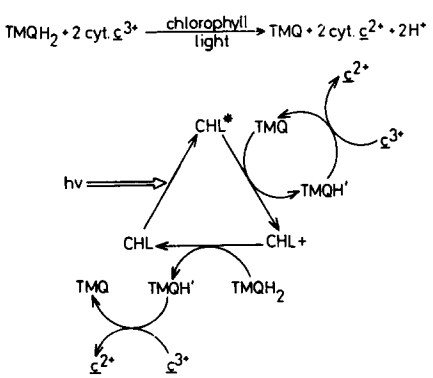

Fig. 4. Catalytic scheme for reduction of cytochrome *c* (adapted from ref. 15).

perature for dipalmitoyllecithin is found calorimetrically at 41 °C, but Fig. 3 shows that when chlorophyll was present in the liposomes, the transition was broadened and moved to lower temperatures, as predicted on the basis of the spectroscopic observations.

The catalytic behaviour of liposomal chlorophyll *b* was followed by the method of Vernon and Shaw [15], using the modified spectrophotometer described above. Aqueous dispersions of chlorophyll catalyse the reduction of cytochrome *c* in the light by various hydrogen donor systems, among which trimethylhydroquinone is the most effective; chlorophyll appears to act by producing quantities of the reactive semiquinone radical in place of the more sluggish quinol, according to the mechanism given in Fig. 4.

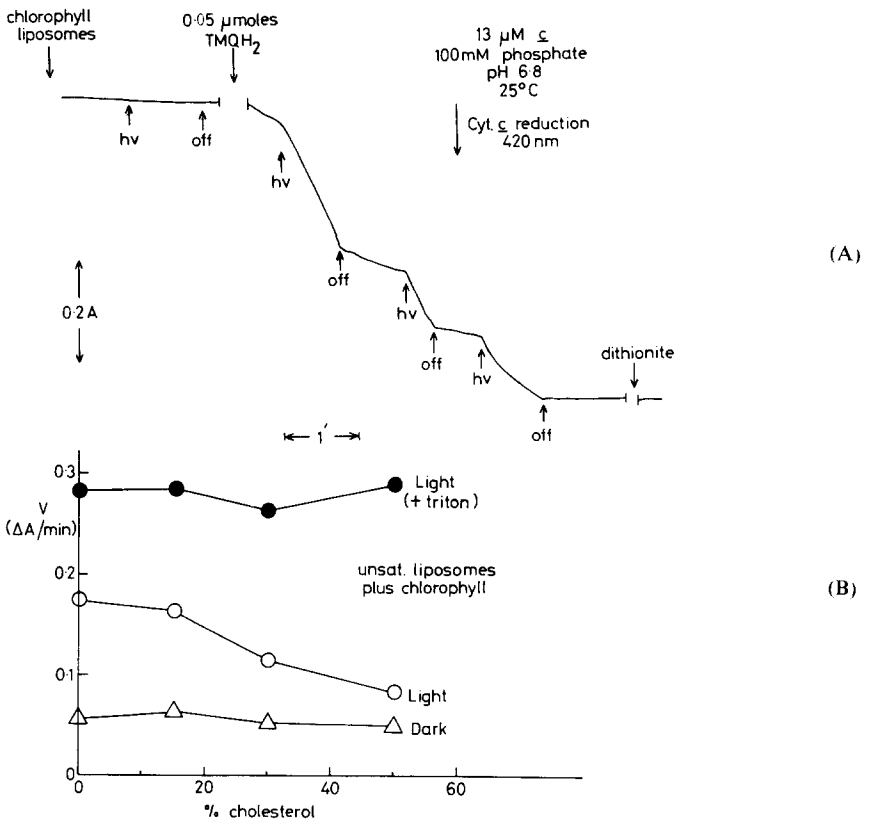

Fig. 5. Photoreduction of cytochrome *c* by trimethylhydroquinone in the presence of chlorophyll-containing liposomes. (A) Time course of cytochrome *c* reduction. A 1.5-ml cuvette was used containing 1.2 ml. of 13 μM *c* in 100 mM phosphate buffer (pH 6.8) at 25 °C. Addition of 0.1 ml suspension of chlorophyll *b* containing liposomes (0.05 μmole chlorophyll *b* and 2.5 μmoles egg phosphatidylcholine/ml) and 5 μl 10 mM trimethylhydroquinone as indicated. Reduction monitored at 420 nm (increasing absorption downwards). "*hv*" indicates switching on light (see Materials and Methods). (B) Effect of increasing amounts of cholesterol in liposomes on the reaction of (A). Rate of light reaction (initial) in ΔA/min in presence and absence of 0.1 % Triton X-100. The liposome preparations contained 0.05 μmole chlorophyll *b*, 2.5 to 1.25 μmoles egg lecithin and from 0–1.25 μmoles cholesterol.

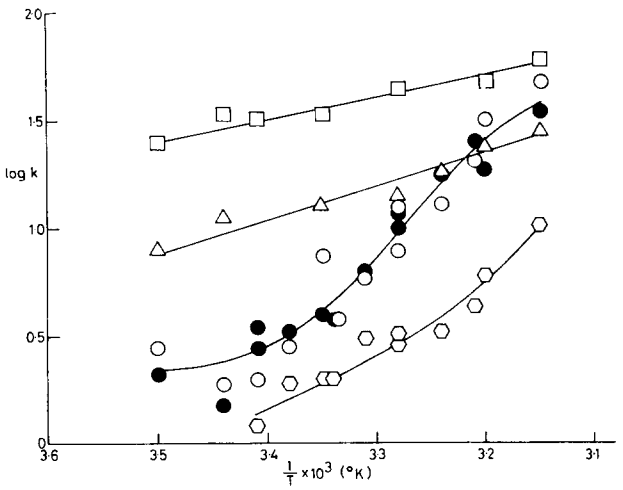

Fig. 6. Arrhenius plots of the reaction of trimethylhydroquinone and cytochrome *c* in the presence of chlorophyll *b* containing liposomes, composed of saturated and unsaturated lipids. Experimental conditions were similar to those of Fig. 5, except for the temperature variation from 13 to 45 °C. 0.1 ml liposomes added to 1.2 ml 13 μM cytochrome *c*. Reaction commenced with 50 nmoles trimethylhydroquinone. □–□, rate in system dispersed with 0.1 % Triton X-100; △–△, egg lecithin liposomes (1.8 μmoles lecithin, 0.07 μmole phosphatidic acid, 0.07 μmoles chlorophyll *b*/ml); ○–○, dipalmitoyllecithin liposomes (uncharged); ●–●, dipalmitoyllecithin liposomes (plus 4 % egg phosphatidic acid); ◇–◇, dark reaction. Conditions (○, ● and ◇): 1.8 μmoles dipalmitoyllecithin, 0.07 μmole chlorophyll *b*, ±0.07 μmole egg phosphatidic acid/ml.

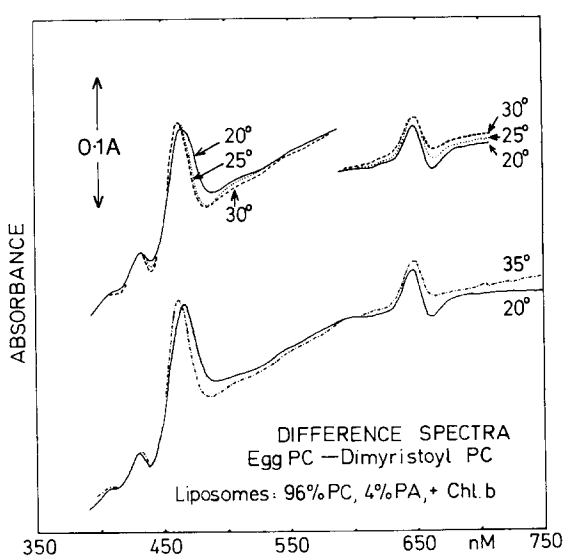

Fig. 7. Difference spectra of chlorophyll *b* in egg lecithin and dimyristoyllecithin liposomes above and below the transition temperature. Conditions as in Fig. 2 using 0.8 μmole dimyristoyllecithin, 0.016 μmole phosphatidic acid, plus 0.016 μmole chlorophyll *b*/ml of liposomes in reference cuvette; 0.8 μmole egg lecithin, 0.016 μmole phosphatidic acid, plus 0.016 μmole chlorophyll *b*/ml in sample cuvette. Upper traces: effect of increasing temperature from 20 to 30 °C. Lower traces: effect of decreasing temperature from 35 to 20 °C. 20 mM phosphate buffer (pH 6.8).

Fig. 5A illustrates the reduction of cytochrome *c* that occurs in the presence of chlorophyll *b* containing liposomes. A similar reaction takes place with chlorophyll *a* liposomes. Cytochrome *c* reduction is absolutely dependent on the presence of added hydrogen donor. Unlike Chapman and Fast [8], we were unable to detect any direct reaction between chlorophyll and cytochrome *c* even when negatively charged liposomes containing 20 % phosphatidic acid were used, which bind cytochrome *c* effectively. However, while the dark reduction of cytochrome *c* by trimethylhydroquinone is first order, the light-induced reduction is zero order until very little cytochrome *c* remains oxidized (Fig. 5A). The rate of the liposome-chlorophyll reaction is about 60 % that of the reaction catalysed by the same amount of chlorophyll dispersed in Triton X-100 solution. Addition of cholesterol to the liposome lipid mixture had the effect of slowing the light catalysed reduction (Fig. 5B).

If liposomes were prepared from dipalmitoyllecithin plus chlorophyll *b* and allowed to cool below their transition temperature they showed almost no photocatalytic activity in the cytochrome *c*–quinol system. As the temperature of the assay is increased, however, photocatalysis is progressively elicited, until at temperatures above 40 °C, the rate is similar to that achieved at the same temperature by a preparation of chlorophyll liposomes from egg phosphatidylcholine. Fig. 6 gives the Arrhenius plots for such systems. Evidently the Triton-dispersed and egg lecithin liposome systems have similar activation energies (lower than that for the dark reaction);

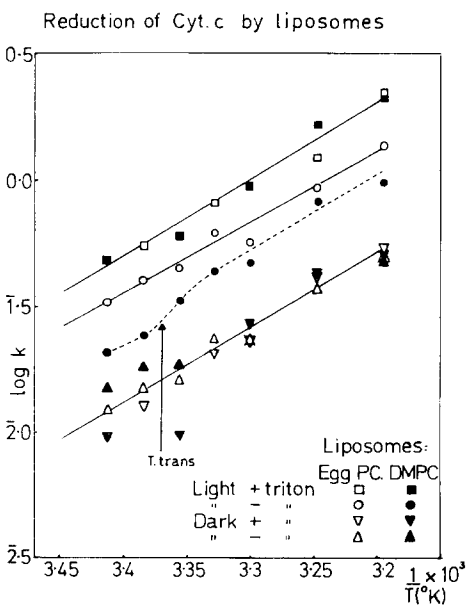

Fig. 8. Arrhenius plots of catalytic activity above and below the transition temperature for chlorophyll *b* containing dimyristoyllecithin liposomes compared with egg lecithin liposomes. Conditions as in Fig. 6, but with dimyristoyllecithin instead of dipalmitoyllecithin chlorophyll *b* containing liposomes as photocatalytic system. ○–○, light reaction; □–□, light reaction + 0.1 % Triton X-100; △–△, dark reaction; ▽–▽, dark reaction + 0.1 % Triton X-100. ○, □, △ and ▽, egg lecithin liposomes. ●–●, light reaction; ■–■, light reaction – 0.1 % Triton X-100; ▲–▲, dark reaction; ▼–▼, dark reaction + 0.1 % Triton X-100. ●, ■, ▲ and ▼, dimyristoyllecithin liposomes.

but the saturated lecithin–chlorophyll system shows a very high "effective activation energy" through its transition region. Apparently the red-shifted chlorophyll (Fig. 2) is much less active than its high temperature counterpart.

When similar experiments were carried out with liposomes formed from dimyristoylphosphatidylcholine instead of dipalmitoylphosphatidylcholine, similar but less striking effects were seen (Fig. 7). A much smaller red shift (Fig. 7) is associated with a very slight increase in activity as the temperature is brought through the transition region (24 °C) (Fig. 8).

As with chlorophyll *b* in dimyristoyllecithin liposomes, chlorophyll *a* in dipalmitoyl lecithin liposomes showed only slight changes an apparent activity with increase in temperature. This correlates with the much smaller effect of temperature on chlorophyll *a* spectrum in dipalmitoylphosphatidylcholine (Table I).

DISCUSSION

The study of chlorophyll behaviour in monolayers was pioneered by Bellamy et al. [16] and followed up by Cherry et al. [10, 17, 18] using chlorophyll-containing black films. In these systems [10], as well as in liposomes [8, 9], the α-peak of chlorophyll *a* was found to lie between 670 and 673 nm. Chlorophyll *b* in bilayer systems [17] had an α-peak at 653 nm, close to the present value of 651 nm for liposomal chlorophyll *b* (Table I).

Cherry et al. [10, 17, 18] and Steinemann et al. [19] have also discussed the location and orientation of chlorophyll within the phospholipid bilayer. Because the relative intensities of the red and blue peaks vary with the plane of polarisation in the incident light, it was concluded [10, 17] that some orientation of the chlorin ring occurred when chlorophyll-containing bilayers were spread on slides. By direct measurements of dichroism at the two peaks, Steinemann et al. [19] found that the angle between the "blue" transition moment and the membrane was about the same for the two chlorophylls (27° for chlorophyll *a*, 29° for chlorophyll *b*) but the corresponding angle for the "red" transition moment was different in the two cases (34° for chlorophyll *a*, 28° chlorophyll for *b*). This would imply that the angle between the plane of the chlorin ring and the membrane might be less for chlorophyll *b* (42°) than for chlorophyll *a* (47°). On the other hand, using an indirect method involving absorption spectroscopy with polarised light Cherry et al. [17] found no difference between the two red transition moments (36.5° for both chlorophylls). They drew the opposite conclusion, namely that the angle between chlorin plane and membrane is greater for chlorophyll *b* (51°) than for chlorophyll *a* (48°). However, their observations indicated changes in peak shape and position with the direction of polarisation, and small changes in measured extinction coefficients give rise to rather large differences in estimated angles.

Steinemann et al. [19] suggest two arrangements for the chlorophyll molecule: (A), the phytyl chain is inserted into the hydrophobic region of the membrane and the chlorin ring projects into the aqueous phase at an angle of 40°–50°. (B), both phytyl chain and chlorin ring are in a hydrophobic milieu, with the chlorin ring bent backwards at an angle of 40°–50° to the membrane surface, and with the reactive and hydrophilic cyclohexanone ring somewhere in the headgroup region.

Our results suggest that the relationship between chlorophyll *b* and the mem-

brane is more sensitive to phase changes than that of chlorophyll *a*. The red shift below the transition temperature may represent a tendency for the membrane to exclude the chlorin ring as it becomes more ordered (cf. the red shift as water is added to solutions of chlorophyll in acetone). Such exclusion could be favoured in the case of chlorophyll *b* because of the extra hydrophilic group (–CHO) on the opposite side of the chlorin ring from the cyclohexanone region. With Steinemann et al. [19] we thus prefer Arrangement B. Our results then predict a decrease in the average angle between chlorin and membrane below the transition temperature.

Cherry et al. [18] have placed the chlorin ring in the head group region, using the very indirect argument that bulk measurements in decane predict an increase in dielectric constant, and hence of membrane capacitance, in the presence of chlorophyll, which is not observed. The close similarity between different lipid systems (lecithin, phosphatidylethanolamine, and phosphatidylserine [19]) as well as the present transition effects, seem to argue against this "third" possible location for the chlorin ring. The smaller effect of temperature on the spectrum and catalytic activity of dimyristoyllecithin liposomes containing chlorophyll *b* (Fig. 7) may be correlated with a more disorderly structure in these liposomes. Nicholls and Miller [20] have reported that dimyristoyllecithin liposomes are more permeable to ions than are dipalmitoyllecithin liposomes.

In all systems examined the cytochrome *c* reduction was dependent on the presence of added quinol. The catalytic act (Fig. 4) appears to involve the production of the more reactive semiquinone radical by chlorophyll. Perhaps the "donor-independent" reduction previously observed [8] was due to very small amounts of endogenous donors in the system employed. But why is chlorophyll in the "frozen" membrane catalytically inactive? Two possibilities exist: (a), that permeability to the quinol/quinone system is restricted and (b), that activated (triplet?) chlorophyll is no longer produced. Although permeabilities decrease sharply below the transition temperature [1, 2], if the chlorophyll is reacting at the membrane surface it should still be accessible. Electron microscope studies (West, J. and Hall, F. J., unpublished) indicate that in the presence of chlorophyll the usual multilayered "onion" structure is disrupted leaving few inacessible interior layers (see also Fig. 1A in ref. 9). Trosper et al. [9] were able to show that the chlorophyll transient is not produced in saturated lecithin solutions. If this is the species required for the quinol–cytochrome *c* catalysis (Ch.* or $Ch.^+$ in Fig. 4) then our results predict that this transient should appear in the saturated liposome–chlorophyll system above the transition temperature.

Spinach chloroplasts have 40 % of their chlorophyll peak at 671 nm, 40 % at 682 nm, and 20 % at 651 nm [21]. Liposomal chlorophyll *a* at 671 nm, and chlorophyll *b* at 651 nm, seem to be in a similar milieu. So far the long wavelength (682 nm) species has not been reproduced in the liposome system, even by raising the chlorophyll/lipid ratio to high values (1 chlorophyll/2 phospholipid molecules).

Both chlorophyll (in the lipid phase) and cytochrome *c* (in the head group region) [22, 23] can be bound to liposomes. So far they cannot however be induced interact directly; carrier systems soluble in both lipid and water are needed. Both interact reciprocally with the membrane : membrane binding alters the potential of cytochrome *c* and its reducibility [22] while cytochrome *c* induces liposome aggregation [23]; similarly the membrane affects the spectrum of chlorophyll, and the chlorophyll affects the transition temperature of the membrane (this paper).

We have been unable to demonstrate any "transmembrane" (e.g. uncoupler-dependent) effects in the chlorophyll–liposome system analogous to the phenomena seen when cytochrome *c* oxidase [24] is incorporated into liposomes. We have also been unable to detect any differences between the spectroscopic appearance of chloroplast from frost-sensitive and frost-resistant plants, or any spectral effects of temperature on such systems in the 0–60 °C range analogous to the effects on chlorophyll in saturated liposomes. However, it is possible that there are membrane transitions in such chloroplasts at environmentally significant temperatures inaccessible to the present apparatus. It is clear that in the artificial system both the spectrum of, and the catalysis by, chlorophyll, depend on mobility of the hydrophobic region of the bilayer. Whether this is also true of photosynthetic activity remains to be seen.

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