Studies of Chlorophyll-Lipid-Water Systems

Abstract. *Preliminary studies of the electronic absorption spectra of chlorophyll molecules in aqueous glycolipid and phospholipid dispersions are reported. The spectra show a shift for the red peak of chlorophyll of some 10 millimicrons to longer wavelength compared with the spectrum of chlorophyll in ether. Photochemical studies were carried out with cytochrome* c *incorporated into the aqueous layers separating the lipid bilayers.*

The ability of membranes to organize enzymes and also energy-transfer molecules, such as carotenes, quinones, and chlorophyll, is presently receiving attention. The detailed arrangement of polar lipid molecules, such as phospholipids, which occurs in the lamellar lyotropic mesomorphic form in water, is considered by some workers to resemble the lipid organization which occurs in many cellular membranes (*1*). This bilayer concept of cell membranes is by no means certain, but it is a useful model at the present time. Phospholipid dispersions, which are considered to give rise to bilayer structures, have recently been used by various workers as model systems of cell membranes, that is, for the study of ion and sugar diffusion properties (*2*). They have also been used to study the mode of action of antibiotics and anesthetics (*3*).

Additional features of these lipid dispersions (shared with monolayer methods) are:

1) It is possible to incorporate sufficient pigment molecules to allow particular chemical reactions to be studied and also to give satisfactory spectroscopic absorbance values; for example, the amount of pigment, such as chlorophyll, which can be incorporated into a single phospholipid bilayer is quite small.

2) It is possible to study the interaction of certain hydrophilic and hydrophobic molecules of biological importance. The hydrophilic molecules can be incorporated into the aqueous layers and the hydrophobic molecules within the lipid layer. Such hydrophobic-hydrophilic interactions are thought to be quite common in biological systems but are difficult to study in isolation.

Here we report preliminary studies of the electronic absorption of chlorophyll molecules in aqueous glycolipid and phospholipid dispersions, and some photochemical studies of these dispersions containing cytochrome *c* in the aqueous compartment.

Pure egg yolk lecithin was prepared by silicic acid chromatography (*4*). Diagalactosyl diglyceride was prepared for us by P. Harries by chromatography (*5*). Chlorophyll *a* was prepared by Harries from the green alga *Plectonema boryanum* and purified on icing sugar columns as recommended by Strain *et al.* (*6*). (This organism does not contain chlorophyll *b*.) A crude fraction of chlorophyll which contained some other pigments was also collected.

Lipid-chlorophyll-water dispersions were prepared by mixing the compounds in chloroform, evaporating off the solvent, then adding the aqueous phase and shaking until a satisfactory dispersion was obtained. The system usually contained 2.2 μmole of lecithin as the dispersant and 3 ml of aqueous phase.

Dispersions were prepared of 0.07 μmole of chlorophyll + 2.2 μmole of lecithin in 1 ml of 0.05*M* tris buffer, *p*H 7.5, containing 0.15 μmole of cytochrome *c*. Aqueous solutions were made anaerobic by repeated evacuation followed by return to atmospheric pressure with nitrogen. All transfers were performed in a dry box in a nitrogen atmosphere and the entire preparation procedure was carried out under green light. Illumination was carried out with a 250-watt photoflood through a 2-inch (5-cm) water filter. To calculate the amount of cytochrome reduced, a value of 19 was used for the difference between the millimolar extinction coefficients of oxidized and reduced cytochrome *c* at 550 mμ. Cytochrome *c* was obtained either from Sigma Chemicals (type 6) or Koch-Light. Coenzyme Q_6 was the product of Sigma Chemicals.

The visible absorption spectra of the crude chlorophyll *a* preparation from *Plectonema* (i) in ether, (ii) dispersed in lecithin–distilled water, and (iii) dispersed in digalactosyl diglyceride–distilled water, were examined. While in ether, the red peak in the chlorophyll spectrum lies at 673 mμ; in both lipid dispersions this band occurs at 683 mμ. In the lipid dispersions the blue peak and the blue satellite peak are also shifted about 10 mμ toward longer wavelengths, and the intensity of the blue peak is greatly decreased (see Table 1).

With a purified preparation (~ 87 percent pure) of chlorophyll *a*, the red peak occurs at 666 mμ in chloroform, and at 673 mμ in the aqueous lecithin dispersion (Table 1). The position of the red peak is unaffected by tenfold changes in concentration of chlorophyll or by substitution of a complex aqueous

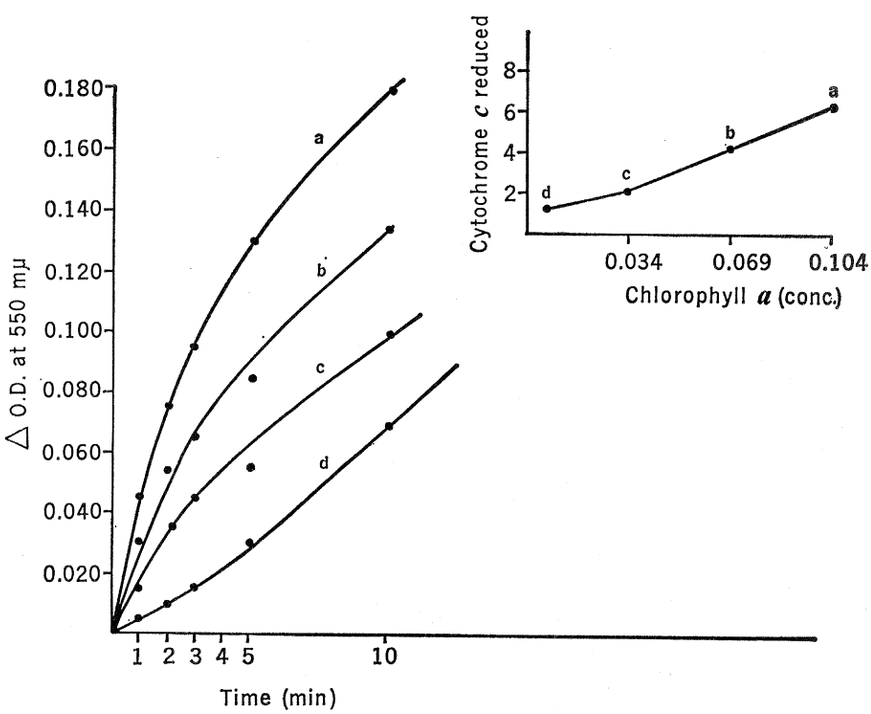

Fig. 1. The dependence of cytochrome *c* reduction on a chlorophyll *a* concentration. Inset shows the relation of the reaction rate to the concentration at zero time. Ordinate of inset: micromoles of cytochrome *c* reduced per micromole of chlorophyll *a*, per hour; abscissa: micromoles of chlorophyll *a* per micromole of lipid.

phase instead of distilled water (see below). There are no absorption bands at longer wavelengths beyond 673 and up to 1000 mμ.

Excitement of the chlorophyll by radiation at a wavelength of 435 mμ leads to fluorescence at 673 mμ whether the chlorophyll is in $CHCl_3$ or in aqueous lecithin dispersions.

Illumination of pure chlorophyll-lipid-cytochrome *c* preparations for several minutes causes a marked photoreduction of cytochrome *c*. The dependence of this reduction on chlorophyll *a* concentration is shown in Fig. 1. The initial reaction rate, when Sigma type 6 cytochrome *c* was used, was approximately 4.1 μmole of cytochrome *c* reduced per micromole of chlorophyll, per hour. With Koch-Light cytochrome *c* the rate was 10.4 μmole of cytochrome *c* reduced per micromole of chlorophyll, per hour. These rates were quite reproducible with the same cytochrome preparation. Sufficient cytochrome was present to saturate the system.

Electron microscope studies of chloroplasts have revealed the existence of an ordered membrane type structure (*7*). It is generally assumed that the membranes are formed of lipid [glycolipids and phospholipids (*8*)] and protein and that the chlorophyll is interspersed perhaps in some well-ordered arrangement, probably with the phytol tail and porphyrin ring extending into the lipid material and the ester and keto linkages in the water phase (*9*).

Many spectroscopic studies have been made of chlorophyll in a variety of organic solvents and it has been observed and commented that these absorption spectra are different from those observed in living cells. Thus chlorophyll *a* in ether or in nujol-benzene shows a strong absorption band at 661 mμ and 662 mμ, but this differs from the band position observed with chlorella suspensions, which is at 675 mμ; the absorption in the latter case extends beyond 700 mμ (*10*). Our results are similar to these observations, and both the pure chlorophyll *a* and the crude chlorophyll in the lipid dispersions show a displacement of the red maximum of absorption of some 10 mμ. The shift of the absorption maximum which we observe may or may not be significant to the organization of chlorophyll in plant systems. As long ago as 1943 Menke (*11*) observed the dichroism of chlorophyll oriented in lecithin, and noted that the artificial dichroism induced was opposite in sign to the one found in chloroplasts. This was confirmed by Goedheer (*12*). The red peak is well known to shift when the environment of the chlorophyll is varied. Thus, when in carbon tetrachloride, the band is at 666 mμ, in pyridine at 671 mμ, and in alanine at 674 mμ (*13*). In the crystalline state the band occurs at 743 mμ when complexed with water, and at 673 mμ in the crystalline state (*14*). The particular feature of our observation is that we have attempted to mimic the situation in the plant by studying the chlorophyll when dispersed in the lipids from the plant itself. Monolayer studies of chlorophyll *a* films have also shown shifts of this order (*10, 15*). However, when chlorophyll molecules are organized in monolayers of phospholipid, such as a phosphatidylethanolamine, the introduction of phospholipid molecules between the pigment reduces the red shift and the band approaches the value observed with chlorophyll *a* in ether solutions (*16*).

Table 1. Visible absorption spectra data for chlorophyll. Abbreviations: Blue S, blue satellite; DGDG, digalactosyl diglyceride.

Solvent	Peak position (mμ)			Red peak half peak width (mμ)	Peak ratios	
	Red	Blue	Blue S		Blue/blue S	Blue/red
			Crude chlorophyll			
Ether	673	430	410	14.0	1.60	1.71
DGDG	683	438	420	20+	1.09	1.44
Lecithin	683	438	420	16.0	1.17	1.38
			Pure chlorophyll a			
Ether	661	427	408	13.6	1.36	1.57
DGDG	671	432	414	19.5	1.01	1.48
Lecithin	673	434	416	16.0	1.04	1.48

The difference between the spectral behavior of pure chlorophyll *a* and the spectral properties of crude chlorophyll may be due to some charge interaction effect with chlorophyll and the other pigments present in this fraction.

Our studies of the chlorophyll-lipid-water system with cytochrome *c* included in the aqueous layers were to assess the potential of such a system. The direct photoreduction of cytochrome *c* by excited chlorophyll is interesting and has not previously been demonstrated. Previous workers (*17*) have shown that cytochrome *c* is not directly reduced by using flash photolysis when chlorophyll *a* is in ethanol. A quinone-catalyzed reduction of cytochrome *c* by chlorophyll *a* does occur in water when chlorophyll and quinone are dispersed by means of Triton X-100, but no attempt directly to reduce cytochrome *c* by excited chlorophyll *a* was made (*18*).

Isolated chloroplasts are capable of reducing cytochrome *c* by a mechanism different from that catalyzed by Hill reagents, some of which are quinones (*19*). The rates for noncatalyzed or "endogenous" cytochrome reduction observed both by these workers and by Vernon and Shaw (*18*) are of the same order of magnitude as we found for direct photoreduction of cytochrome *c* by chlorophyll *a* in lipid-water dispersions.

Our results, although preliminary, are interesting and provocative and suggest that further studies of model chlorophyll-lipid systems may be worthwhile.

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References and Notes

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20. This work was done while P.G.F. (Insect Pathology Research Institute, Sault Ste. Marie, Ontario, Canada) was on a postdoctoral transfer of work to Unilever Research Laboratory.

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