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Chlorophyll *a*-containing liposomes*

Several investigators have suggested that phospholipid suspensions in aqueous salt solutions, or liposomes, are useful models for study of the biophysical properties of membrane lipids[1,2]. CHAPMAN AND FAST[3] have employed similar preparations incorporating chlorophyll to investigate the ability of the pigment in a membrane-like environment to reduce added cytochrome *c*. These preparations are better suited to spectroscopic studies than are fragile bilayers, because a liposome suspension of sufficient optical absorbance is easily obtained[3].

Models consisting of chlorophyll dispersed in phospholipid are more appropriate biologically than monolayers or dried films containing chlorophyll. We have also studied liposomes containing chlorophyll *a* and find that the pigment is well dispersed in these structures in the presence of unsaturated lipid. In this case, reversible formation of chlorophyll *a* triplet is observed upon flash illumination.

Dispersions were prepared following the procedure of BANGHAM *et al.*[4], with the exception that total lipid concentration was roughly 5 μmoles/3 ml, or 1/3 that previously used. Suspensions were degassed and blanketed with argon before being swirled 1 to 2 h. All transfers of material containing egg phosphatidyl choline were preformed in a nitrogen atmosphere at room temperature, and all experiments involving chlorophyll *a* were done in subdued light or in the dark.

Suspensions were prepared from chlorophyll *a* purified by sugar column chromatography, and synthetic dipalmitoyl phosphatidyl choline (DPC) (Fluka), DPC *plus* glycerol mono- or dioleates (Hormel Institute, Austin, Minn.), or purified egg phosphatidyl choline (Supelco, Bellefonte, Pa.). Aqueous suspending media contained 0.11 M NaCl, 0.12 M ammonium acetate, or 1 % (w/v) ammonium molybdate, all buffered with approx. 0.03 M K phosphate at pH 7.1. Pigment concentrations were adjusted so that a suspension of 1 cm light path had an absorbance of 0.4 to 0.8 at the chlorophyll red maximum and a chlorophyll:lipid ratio of roughly 1:30 to 1:70.

Chlorophyll *a* appeared to be dispersed in these liposome preparations. Absorption spectra recorded on a Cary 14 spectrophotometer with a scattered-transmission attachment had a red maximum at 670 nm, and peaks at approx. 438 and 418 nm in the Soret region. In addition to the red shift from 665 nm in chloroform solution, the blue maximum at 438 nm was somewhat depressed and the blue shoulder enhanced, with respect to the chlorophyll *a* solution spectrum. This change in the Soret region is probably caused by association of the pigment with phospholipid, because it occurs also in solutions of chlorophyll *a* and egg phosphatidylcholine in chloroform. Only negligible amounts of chlorophyll *a* could be suspended in buffered salt solutions in the absence of phospholipids. Further, the absorbance of aged suspensions (longer than 24 h) decreased as particulate matter settled out, and chlorophyll was noticeably concentrated in the lipid sediment. These facts further indicate that pigment was associated with the liposomes.

All liposome preparations were examined by electron microscopy. They were visualized usually by negative staining with buffered ammonium molybdate or potas-

Abbreviation: DPC, dipalmitoyl phosphatidyl choline.

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sium phosphotungstate. To corroborate sizes and shapes, some samples were also lyophilized on a grid and shadowed with platinum–carbon.

Images of both small vesicles and of layered aggregates with a concentric lamellar appearance were observed, similar to structures previously seen in smectic mixtures

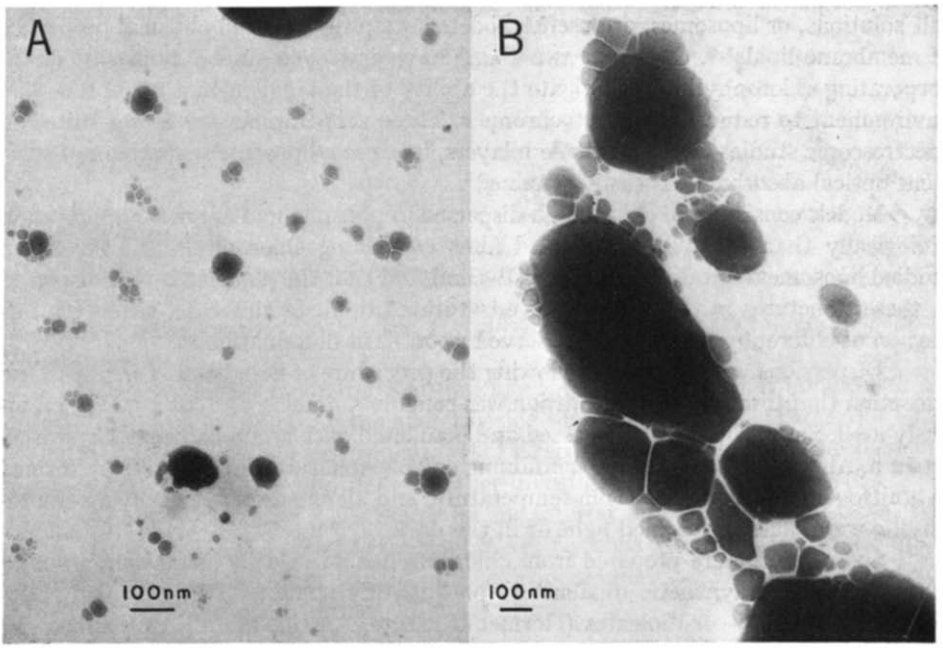

Fig. 1. (A) Chlorophyll *a*–egg lecithin liposomes are mostly small vesicles (10–50 nm), visualized by the liquid they retained on forming in 1 % ammonium molybdate, approx. 0.3 M K-phosphate, pH 7.1. (B) Detail of a few larger vesicles indicates that each is an intact bounded unit.

of pure and mixed phospholipids[5,6]. These liposomes seem to be vesicles, heterogeneous in size, bounded by a membrane, enclosing a liquid-filled volume. Fig. 1 shows representative material from a chlorophyll *a*–egg phosphatidyl choline dispersion in buffered ammonium molybdate. Placed on a grid, the bulk stain often drained off the supporting carbon film, but remained inside the vesicles. These are mostly small (Fig. 1a). There are also areas of larger aggregates showing the vesicles as membrane-bound droplets adhering to or overlapping each other (Fig. 1b). The liposomes pictured here were photochemically active as described below.

Degassed liposome suspensions containing chlorophyll *a* and unsaturated lipids exhibited light-induced transient absorption changes. Using the flash kinetic spectrophotometer[7], we detected a rapidly decaying absorption decrease in the Soret region (Fig. 2a). The spectrum of the absorption change throughout the blue is shown in Fig. 2b. This spectrum, with the negative peak and shoulder in the Soret region, is similar to those which have been observed in chlorophyll *a* solutions by LINSCHITZ AND SARKANEN[8] and in dried egg phosphatidyl choline–chlorophyll *a* films by KELLY[9]. These workers have attributed the transient to formation of chlorophyll *a* triplet. The decay time of the chlorophyll liposome signal, $t_{1/2}$ is approx. 0.5 msec, also indicates that the transient we observed was caused by triplet formation. Furthermore,

the transients were only detected when liposomes contained unsaturated lipid. Chlorophyll *a* may be poorly dispersed in saturated lipids such as DPC at an interface[10]. The triplet state would be quenched at the resulting high pigment concentrations[11], thus no transients should be observed.

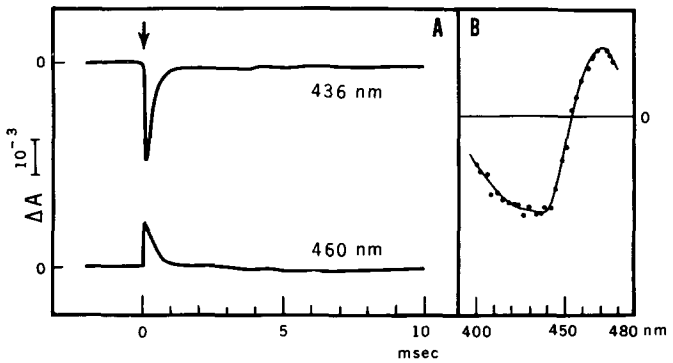

Fig. 2. (A) Transient absorption changes observed at 436 and 460 nm when chlorophyll *a*–egg phosphatidyl choline liposomes in 1 % buffered ammonium molybdate were irradiated with 20 μsec flashes (650–750 nm). Absorbance of suspension was 0.41 at 670 nm. (B) Spectrum of the absorbance changes in the blue region. Sample and experimental conditions as in (A).

Chlorophyll *a* is most probably associated with unsaturated lipid in liposome suspensions. The pigment appears to be in the lipoidal lamellar phase of the dispersions. In this condition chlorophyll *a* is capable of undergoing reversible photoinduced triplet formation. Such a model is highly relevant to the environment of chlorophyll in chloroplast lamellae, which contain large amounts of unsaturated structural lipid[12]. Chlorophyll-containing liposomes constitute a system well suited to further investigation of the interactions of the pigment with redox reagents and proteins involved in photosynthetic reactions.

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