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SIMULATION OF GRANA STACKING IN A MODEL MEMBRANE SYSTEM

MEDIATION BY A PURIFIED LIGHT-HARVESTING PIGMENT-PROTEIN COMPLEX FROM CHLOROPLASTS

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Summary

An isolated light-harvesting pigment-protein complex contains polypeptides which bind chlorophyll *a* and *b*. The individual complexes can be purified from detergent-solubilized membranes. The isolated light-harvesting complex, when dialyzed to remove detergents, was examined by freeze-fracture electron microscopy. The material consisted of planar sheets of 80-Å subunits which interacted via an edge-to-edge contact. Addition of cations caused the planar light-harvesting complex sheets to become tightly appressed in multilamellar stacks, with distinct subunits still visible within each lamellar sheet. A transition of particle organization from random to crystalline occurred in parallel with the cation-induced lamellar association. Treatment of the dialyzed light-harvesting complex subunits with low levels of the proteolytic enzyme trypsin removed a 2000 molecular weight segment of the major polypeptide of the light-harvesting complex and blocked all subsequent cation-induced changes in structural organization of the isolated light-harvesting complex lamellar sheets.

To gain further evidence for mechanisms of cation effects upon the organization of the light-harvesting complex in native membranes, the light-harvesting complex was incorporated into uncharged (phosphatidylcholine) lipid vesicles. The protein complexes spanned the lipid bilayer and were arranged in either

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Abbreviations: Chl, chlorophyll; LHC, light-harvesting complex; PS I, Photosystem I; PS II, Photosystem II; C-XXX, chlorophyll form absorbing at XXX nm; F-XXX, chlorophyll form fluorescing at XXX nm; EDTA, ethylenediaminetetraacetic acid; 'low-salt', 20 mM monovalent cation.

a random pattern or in hexagonal crystalline lattices. Addition of either monovalent or divalent cations to 'low-salt' (20 mM monovalent cation) vesicles containing light-harvesting complex caused extensive regions of membrane appression to appear. It is concluded that this cation-induced membrane appression is mediated by surface-exposed segments of the light-harvesting complex since (a) phosphatidylcholine vesicles themselves did not undergo cation-induced aggregation, and (b) mild trypsin digestion of the surface-exposed regions of the light-harvesting complex blocked cation-induced lamellar appression. The particles in the appressed vesicle membranes tended to form long, linear arrays of particles, with occasional mixed quasi-crystalline arrays with an angular displacement near 72°. Surface-mediated interactions among light-harvesting complex subunits of different membranes are, therefore, related to changes in structural organization and interaction of the particles within the lipid phase of the membrane.

Numerous previous studies have implicated the involvement of the light-harvesting complex in mediating grana stocking in intact chloroplast membranes. The data presented herein provide a simulation of the membrane appression phenomena using a single class of chloroplast-derived membrane subunits. The data demonstrate that specific surface-localized regions of the light-harvesting complex are involved in membrane-membrane interactions.

Introduction

Grana stacks are predominant and characteristic structural features of higher plant chloroplasts. In these stacks, 2—20 or more disc-shaped thylakoids lie appressed in a structure resembling a stack of coins. Questions concerning the functional significance of this pattern of membrane organization have been raised for a number of years, these questions have led to a search for the component of the membrane mediating the stacking process. The most likely candidate at the present time is light-harvesting pigment-protein complex (LHC) which has been observed to appear in parallel with stacking during chloroplast greening [1—3], is depleted or modified in various grana-deficient mutant species [4—6], and is modified by proteolytic enzymes in parallel with the loss of grana stacking [7—10]. It is now widely accepted that chloroplast grana stacking can be reversibly lost by suspending isolated thylakoids in 'low-salt' solutions (20 mM monovalent cations or less). Readdition of low concentrations of divalent cations (2—5 mM Mg^{2+}) or higher concentrations of monovalent cations (100—150 mM Na^+ or K^+) will restore membrane appression in these 'low-salt' samples [11,12].

A number of research laboratories have correlated salt effects on membrane appression with cation-induced changes in excitation energy distribution between Photosystem I and II [2,5,9,10,13—15]. In 'low-salt' chloroplasts, absorbed excitation energy is preferentially distributed to Photosystem I [16]; addition of either monovalent or divalent cations (in amounts that would restore grana stacking) causes a change in the distribution of absorbed excitation energy in favor of Photosystem II. This regulation of excitation energy distribution is though to be important in controlling the quantum efficiency

of the light reactions of photosynthesis [15]. Several studies have demonstrated that the LHC is required for control of this energy distribution process [2,5,17].

It has been suggested that the grana stacking process, per se, is intimately involved in regulation of the absorption processes involved in light capture and energy distribution between Photosystem I and II [9,17]. We have previously argued that cations alter the interaction among chlorophyll-bearing protein subunits of the chloroplast membrane [18], and that a surface-exposed segment of the light-harvesting complex is responsible for the cation-mediated changes [10,19].

The previous studies which have implicated the involvement of the LHC in both cation-induced structural changes of the plastid and functional alterations in light-harvesting properties have been based on correlative data. We now wish to present more direct analysis of the cation-induced structural changes of the LHC. For these experiments a method for isolation and purification of the LHC is utilized, and techniques are described for incorporation of the LHC in lipid vesicles such that direct studies on structural changes induced by cations could be studied in a membrane system model containing only this one component. The structural organization of the LHC particles in the model membranes will be discussed in terms of its role in the chloroplast membrane as the 'stacking factor', and in terms of mechanisms by which cations can regulate excitation energy distribution processes. Preliminary results of these studies were previously reported [20].

Materials and Methods

LHC preparation and characterization. The LHC of pea chloroplasts was solubilized from EDTA-washed chloroplasts using Triton X-100 and was separated from other membrane components by a sucrose gradient centrifugation according to the procedures of Burke et al. [19], with the exception that the Triton X-100 on the gradient was adjusted to 0.05%. The highly fluorescent chlorophyll-containing band removed from near the top of the gradient, as described by these authors, and was precipitated using 5 mM $MgCl_2$. LHC from six different preparations was combined and suspended in 5 mM EDTA, pH 7.8. The LHC particles were then pelleted by centrifugation at 41 000 × *g* for 10 min. The EDTA-washed LHC was subsequently dialyzed 12 h at 4° C against several changes of 1 mM NaCl in Tricine/NaOH (pH 7.8).

In some experiments, aliquots of the washed, dialyzed LHC were resuspended at room temperature to a final concentration of 200 μg Chl/ml in the dialysis buffer solution and digested with the proteolytic enzyme trypsin. Trypsin (from bovine pancreas, type III, 120 000 units/mg protein, Sigma Chemical Co., St. Louis, MO) was added to a final concentration of 0.5 μg/ml. Trypsin digestion was stopped after 5 min by the addition of a twenty-fold excess of trypsin inhibitor (from soybean, type I-S, Sigma Chemical Co.). The LHC was subsequently pelleted by centrifugation at 41 000 × *g* for 10 min at 4° C. LHC which had been incorporated in vesicles prior to trypsin digestion was treated in a similar manner.

For analysis of sample purity and LHC protein characteristics, slab gel SDS-polyacrylamide gel electrophoresis was carried out as described by Steinback et al. [10]. Structural analysis of the samples used freeze-fracture techniques for electron microscopy. Samples were prepared and analyzed as previously described [21] using a Hitachi H-300 electron microscope for photography of replicas. Cation-induced changes in 180° light scattering in the samples was monitored by measuring absorbance changes at 550 nm in samples containing 10—15 μg Chl/ml using an Aminco DW-2 spectrophotometer (American Instruments Co., Silver Springs, MD 20901).

Vesicle preparation. Vesicles were prepared by cholate, Sephadex G-75 chromatography techniques described by Brunner et al. [22]. Vesicles were collected using a Brinkman fractionator and subsequently dialyzed under N_2 for 12 h against several changes of 1 mM NaCl, 1 mM Tricine/NaOH, pH 7.8. The dialyzed vesicles were stored under N_2 at 4° C unti use. Phosphatidylserine (type II-2) and phosphatidylcholine (type III-E) were obtained from Sigma Chemical Co.

Absorption spectra were acquired using an Aminco DW-2 spectrophotometer. Samples were suspended to 10 μg Chl/ml in solutions described in the text. Fluorescence emission spectra were obtained by the dual-channel ratiometric acquisition method using a System 4000 scanning polarization spectrofluorimeter (SLM Instruments, Urbana, IL 61801). Multiple spectra [5] were recorded and averaged; uncorrected spectra are presented. Samples containing 0.5—1.0 μg Chl/ml were assayed in 50% glycerol at 77 K.

Results

Characterization of the LHC particles

The light-harvesting pigment-protein complex (LHC) preparation used in the current investigation was similar to that previously described [19] with the following exceptions. Only $MgCl_2$ was used to precipitate the gradient-separated material; the purified sample of LHC was washed with EDTA to remove bound Mg^{2+}, and the sample was subsequently dialyzed against 20 mM Tricine/NaOH ('low-salt', pH 7.8) to yield a purified pigment-protein sample which was depleted of residual detergent.

An SDS slab gel electrophoresis pattern of isolated LHC as compared to control membranes is shown in Fig. 1. It was found that the isolated complex contained polypeptides of 26—30 kilodaltons. This portion of the gel is shown in enlarged scale as an inset at the bottom of Fig. 1; corresponding multiple bands of the LHC in native membranes and the purified complex can be seen. Also shown in Fig. 1 is the polypeptide profile after trypsin treatment of isolated LHC. The protease modified the higher molecular weight polypeptides such that all polypeptides migrated at 26 kilodaltons. These changes were consistent with trypsin modification of LHC in chloroplast membranes where the same pattern of polypeptide modifications was previously observed [10].

The structure of purified LHC was examined under various conditions by freeze-fracture techniques. LHC which had been EDTA washed, then dialyzed vs. 1 mM NaCl plus 1 mM Tricine/NaOH, pH 7.8, is shown in Fig. 2A. The predominant feature of these preparations was the presence of sheet-like

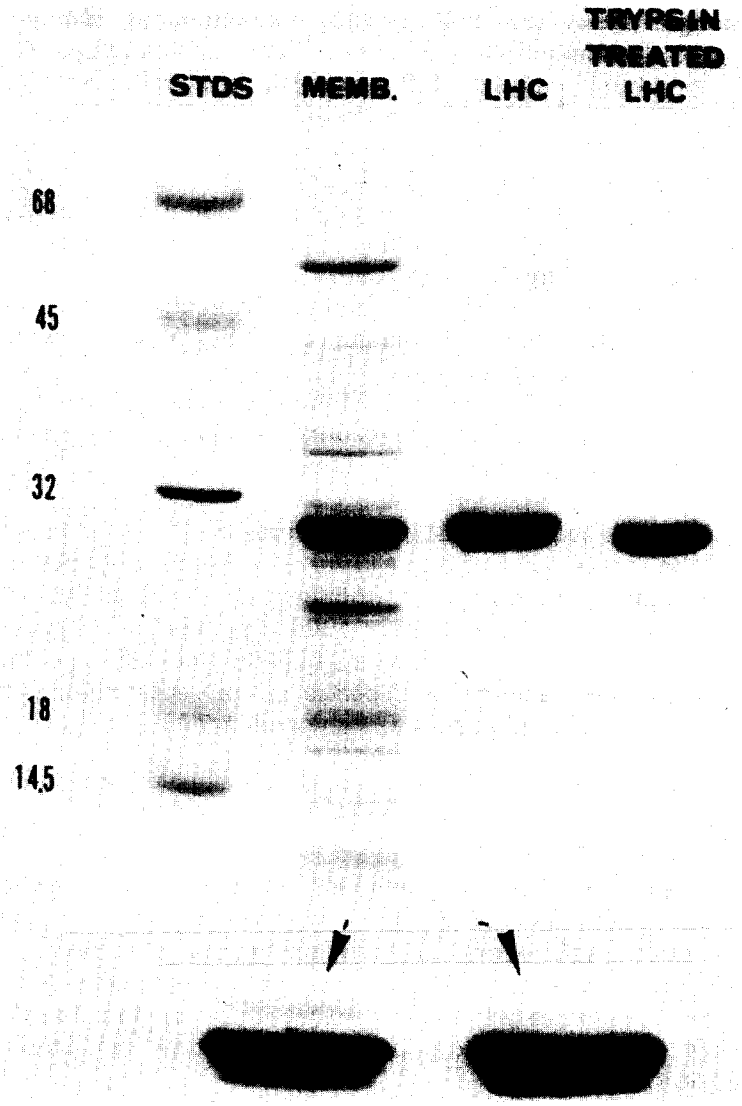


Fig. 1. 10—18% SDS-polyacrylamide slab gel of chloroplast membranes (MEMB); isolated LHC and LHC after trypsin treatment for 10 min (0.5 μg/ml trypsin vs. 200 μg LHC Chl/ml). The polypeptide profile shown in the lower (inset) portion of the figure reveals multiple polypeptide bands in the isolated LHC and in chloroplast membranes. Molecular weight standards (STDS) usd are: bovine serum albumin (68 kilodaltons), ovalbumin (45), carbonix anhydrase (32), β-lactoglobulin (18), and lysozyme (14.5).

aggregates of randomly organized particulate subunits. The lamellar sheets occasionally interconnected, but were not appressed. A cross-fracture of one region of the lamellar sheets is shown in Fig. 2B; the individual subunits making up the sheet can be seen as beads interacting in an edge-to-edge fashion. The individual subunits in these cross-fractured lamellar preparations were found to be 60—100 Å in diameter. We interpret the data to indicate that each of these subunits represents an individual light-harvesting pigment-protein complex (LHC).

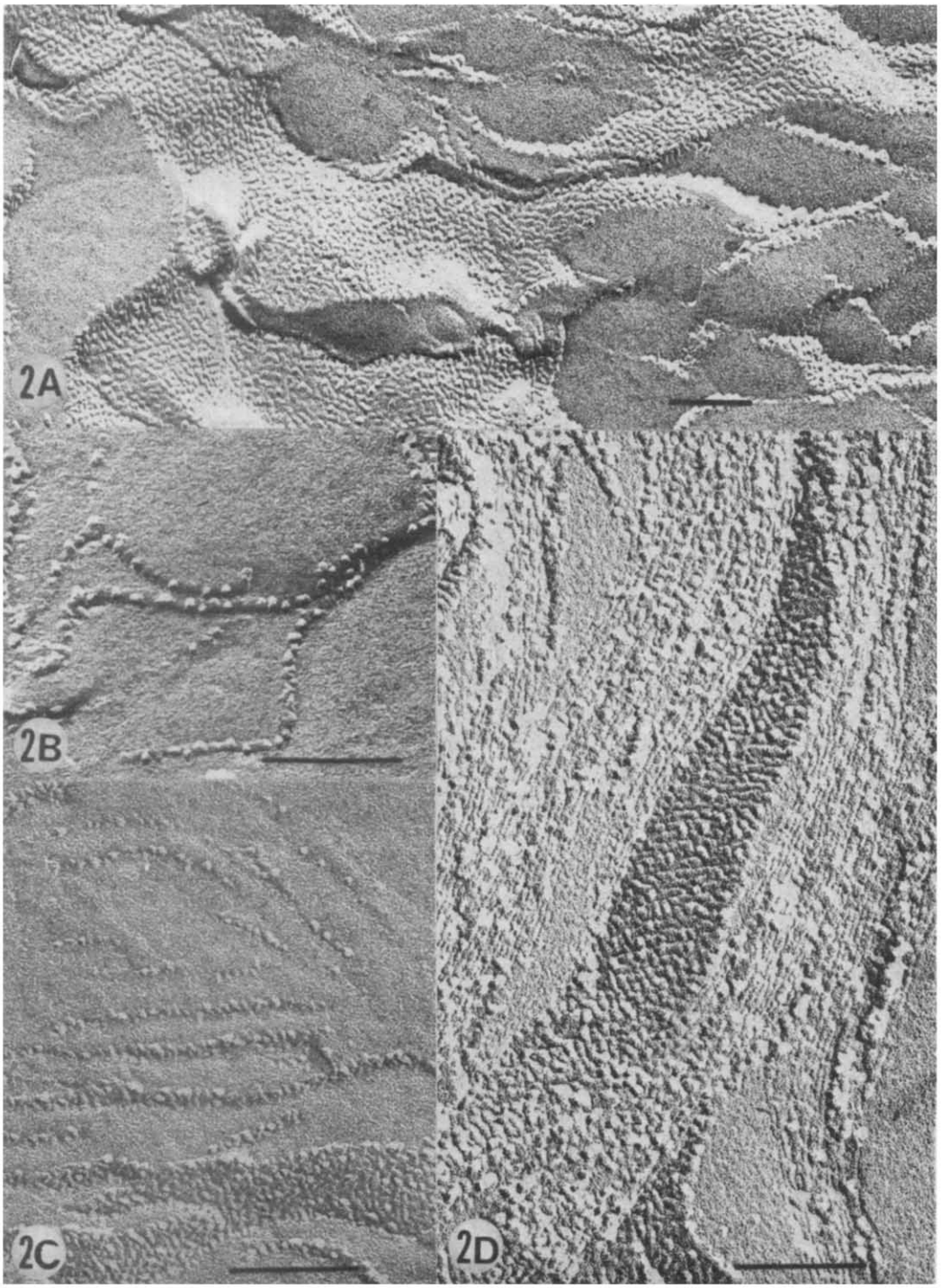


Fig. 2. Electron micrographs of freeze-fractured, isolated LHC. (A) LHC in 1 mM Tricine/NaOH, 1 mM NaCl, after dialysis to remove detergents. (B) Cross-fracture of LHC prepared as in (A). (C) LHC, pretreated with 0.25 μg trypsin vs. 100 μg LHC Chl/ml, then fractures in the presence of 1 mM Tricine/NaOH, 1 mM NaCl, 5 mM $MgCl_2$. (D) LHC in 1 mM Tricine/NaOH, 1 mM NaCl, 5 mM $MgCl_2$. Bars equal 0.1 μm.

When 5 mM $MgCl_2$ was added to the isolated, dialyzed LHC preparations, an increase in turbidity and light scattering of the sample was evident by visual observation. Previous studies using thin sectioning for electron microscopy [19] have indicated that this divalent cation-induced response was due to an aggregation of the LHC. This is more clearly shown by freeze-fracturing techniques in Fig. 2D. Upon addition of $MgCl_2$, appression of the lamellar sheets into multiple stacks of lamellae is revealed by the lines formed where the lamellae have been cross-fractured. In addition, on the lamellar faces shown in tangential fracture, the particles has rearranged into crystalline lattices concomitant with the LHC sheet appression.

The structure of trypsin-treated LHC was examined under conditions identical to those described above. Trypsin-treated LHC resuspended in 20 mM Tricine/NaOH was found to contain particles that were 60—100 Å in diameter. The particles were arranged in sheets similar to those seen in 'low-salt' LHC. Fractures of trypsin-treated LHC in the presence of 5 mM $MgCl_2$ (Fig. 2C) revealed particles and organization identical to LHC in 'low-salt'; i.e. trypsin modification blocked all cation-induced changes in particle orientation and all lamellar appression but did not change the basic structure of the LHC particles to an extent detectable at our level of resolution.

Since addition of $MgCl_2$ to isolated LHC preparations induced aggregation of the lamellar material, we have used changes in 180° light scattering to characterize the cation concentration requirement for this process. Results of monitoring absorbance changes at 550 nm (as a method of determining LHC aggregation) are shown in Fig. 3. LHC which had not been trypsin-treated showed increases in A_{550} in the presence of either NaCl or $MgCl_2$. The salt concentration where half-maximal effects were observed was 2.5 mM for $MgCl_2$ and 100 mM for NaCl. It was noted that no NaCl concentration used could induce as large an A_{550} change as could 10 mM $MgCl_2$. LHC which had

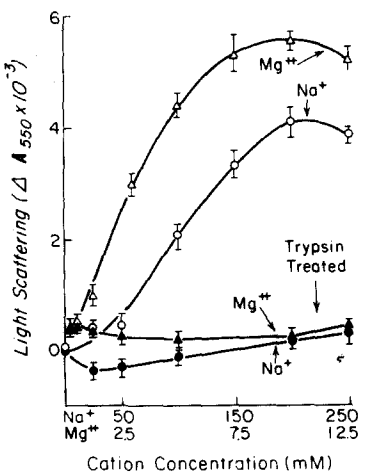
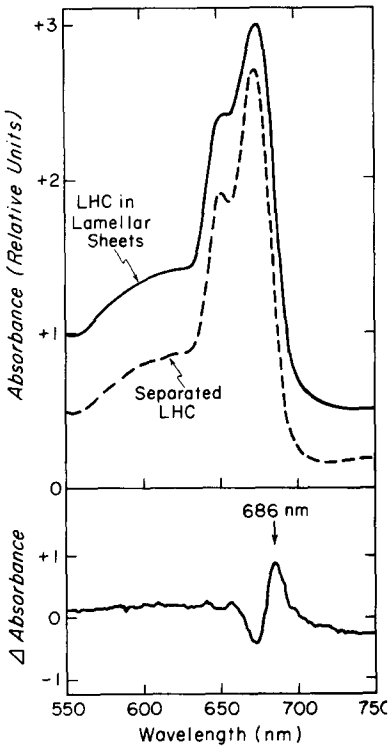


Fig. 3. Light-scattering changes (ΔA_{550}) were monitored for samples containing LHC (10 μg Chl/ml). NaCl or $MgCl_2$ was added to samples then incubated 30 min prior to assay. LHC samples were incubated in the presence or absence of trypsin as described in Fig. 1 prior to addition of cations.



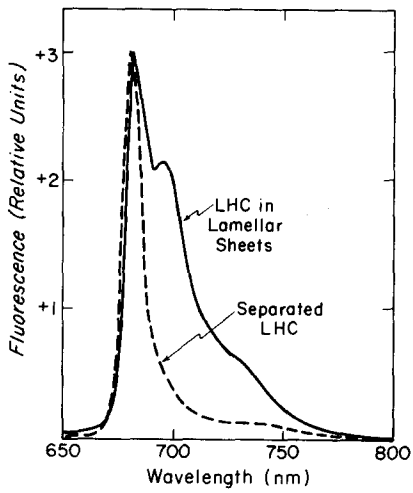


Fig. 4. 20°C absorption spectrum of LHC solvated (5 mg octyl glucopyranoside, 5 mg digitonin/1 mg chlorophyll) or in lamellar sheets as indicated. The difference spectrum (LHC in lamellar sheets minus solvated LHC) is shown in the lower portion of the figure.

Fig. 5. 77 K fluorescence emission of solvated LHC (see Fig. 4) and LHC in particle sheets. Excitation wavelength was 440 nm (8 nm slit) and emission was collected using a 1 nm slit. Solvated LHC exhibited increased total fluorescence yield and a relative decrease in fluorescence at 695 nm.

been trypsin-treated revealed only small cation-induced changes in A_{550} (Fig. 3); this is consistent with a lack of membrane appression observed by freeze-fracturing.

The fact that the LHC can be obtained in different structural states ('solvated', separate complexes with low levels of bound detergents; subunits arranged in sheets after dialysis removal of detergents; appressed sheets in the presence of salts) allowed investigation of how these states influence pigment characteristics. The absorption spectrum of detergent-solvated LHC and dialyzed LHC are shown in Fig. 4; a difference spectrum (dialyzed minus solvated LHC) is shown in the lower portion of the figure. The sheet-like aggregates of LHC are clearly enriched in a long wavelength form of Chl *a*. Analysis of fluorescence emission spectra at 77 K of these same samples is shown in Fig. 5. It was observed that dialysis-induced subunit aggregation decreased the fluorescence yield of the LHC; for comparison in the figure, however, the two spectra are normalized at the peak emission. The appearance of a long-wave form of Chl *a* in the aggregated sheets (Fig. 4) may correspond to increased importance of a chlorophyll form fluorescing at 695 nm (Fig. 5).

It should be emphasized that the samples used for spectral comparisons in Figs. 4 and 5 were identical except for structural state; the differences observed represent spectral alterations caused by differences in subunit interactions and/or changes in local hydrophobic environments around or within each structural unit.

LHC in vesicles

In order to examine cation-mediated LHC aggregation under conditions similar to in vivo conditions, we incorporated LHC into vesicles of various lipid types. Incorporation was accomplished by mixing dialyzed LHC with vesicles in the presence of 2 mM $MgCl_2$ for 60 min. This mixture was then loaded onto 0.5—2.0 M sucrose gradients and centrifuged at 100 000 × *g* for 20 min. Incorporation of LHC into vesicles decreased the pigment-protein's buoyant density; the position of the pigmented material on the gradients was therefore utilized as a direct measure of incorporation. Various amounts of lipid vesicles were titrated vs. a constant amount of LHC to determine optimal LHC/lipid ratios. Fig. 6 shows the decrease in buoyant density of LHC in vesicles (tubes 2—4) compared to free LHC (tube 1). Suboptimal LHC/lipid ratios resulted in a broad green band extending over the middle of the tube (tube 2); this material represents a range of LHC/lipid aggregates of mixed ratios. Optimal LHC/lipid ratios were assumed when a tight green band with high buoyant density was obtained. For most experiments reported herein,

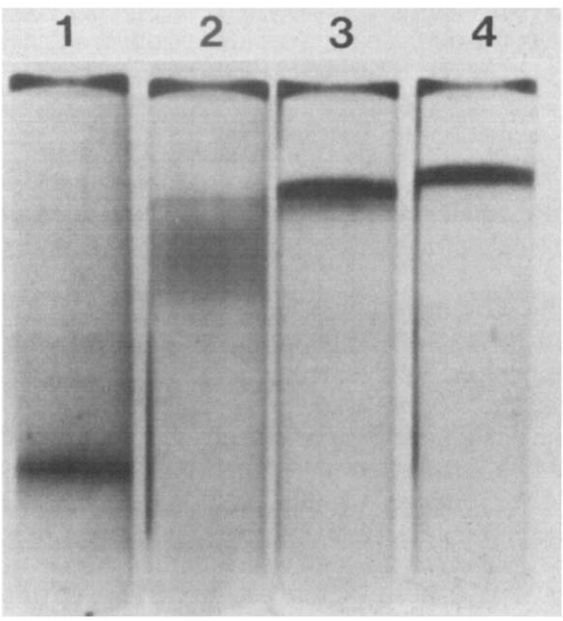


Fig. 6. Sucrose gradients (0.1—1.0 M) showing the relative position of isolated, dialyzed LHC (tube 1) and LHC incorporated into various amounts of lipid (tubes 2—4), after centrifugation in sucrose gradients at 100 000 × *g* for 20 min. LHC/lipid ratios are 15 μg LHC Chl/0.1 mg lipid (tube 2), 15 μg LHC Chl/0.5 mg lipid (tube 3), and 15 μg LHC Chl/1.0 mg lipid (tube 4).

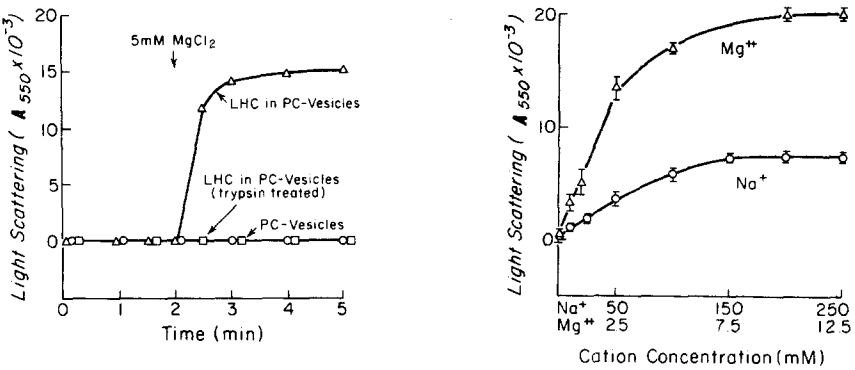


Fig. 7. Time course of light-scattering changes monitored at A_{550}. A light-scattering increase, induced by the addition of 5 mM $MgCl_2$ was observed for LHC incorporated into phosphatidylcholine vesicles, but not for LHC incorporated into phosphatidylcholine vesicles and then trypsin-treated (see Fig. 1), or for phosphatidylcholine vesicles alone.

Fig. 8. Light-scattering changes (A_{550}) induced by NaCl or $MgCl_2$ when added to phosphatidylcholine vesicles containing LHC. Chlorophyll concentrations of solutions used for assays were 15 μg Chl/ml.

LHC/lipid ratios of 15 μg Chl/0.5 mg lipid were utilized (shown in gradient 3, Fig. 6).

In initial experiments, LHC was inserted into vesicles containing phosphatidylserine and phosphatidylcholine (phosphatidylserine: phosphatidylcholine, 1 : 1) as described above. Preparations of this type were centrifuged and then dialyzed vs. 1 mM NaCl, 1 mM Tricine/NaOH, pH 7.8. Particulate subunits were observed in the vesicles when these were examined by freeze-fracture, whereas lipid vesicles containing no pigment-protein showed smooth fracture faces. Addition of 5 mM $MgCl_2$ to LHC-containing vesicles caused aggregation; in control experiments, however, LHC-free vesicles also aggregated in the presence of $MgCl_2$. This could be detected either in freeze-fracture analysis or by monitoring light-scattering changes at 550 nm.

Since there were effects of cations on the organization of charged vesicles, we could not utilize these for testing the specific involvement of LHC in the cation-mediated structural event. We therefore used vesicles of purified egg phosphatidylcholine; these lipid vesicles did not aggregate when incubated with 5 mM $MgCl_2$ (Fig. 7). In contrast, phosphatidylcholine vesicles containing LHC showed a large light-scattering increase (indicating aggregation) upon addition of $MgCl_2$ (Fig. 7). Pretreatment of LHC in phosphatidylcholine vesicles with trypsin prior to $MgCl_2$ addition blocked all cation-induced changes. (Examination of the polypeptides of the vesicle-incorporated LHC before and after trypsin treatment by gel electrophoresis showed that trypsin had identical effects on the incorporated pigment-protein as were observed with the dialyzed, free LHC; Fig. 1). The cation concentration requirements for eliciting the aggregation of LHC/phosphatidylcholine vesicles are shown in Fig. 8. Half-maximal effects were observed at 1.75 mM $MgCl_2$ or 50 mM NaCl.

Examination of the LHC incorporated into phosphatidylcholine vesicles by freeze-fracture techniques for electron microscopy revealed the presence

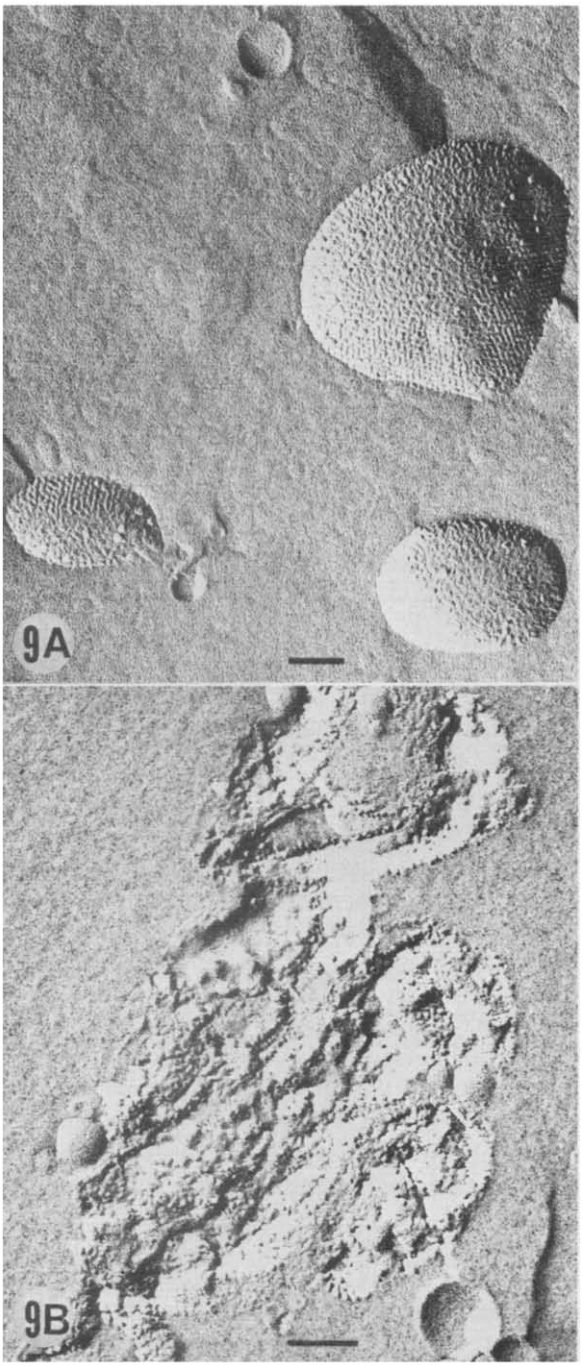


Fig. 9. Electron micrograph of freeze-fractured LHC/phosphatidylcholine vesicles. (A) Single lamellar phosphatidylcholine vesicles containing LHC in the presence of 1 mM Tricine/NaOH, 1 mM NaCl. (B) Vesicle populations as in (A) but after addition of 5 mM $MgCl_2$; massive clumping caused by vesicle aggregation results in lack of a clear fracture plane and thus in lack of clear visualization of particle organization.

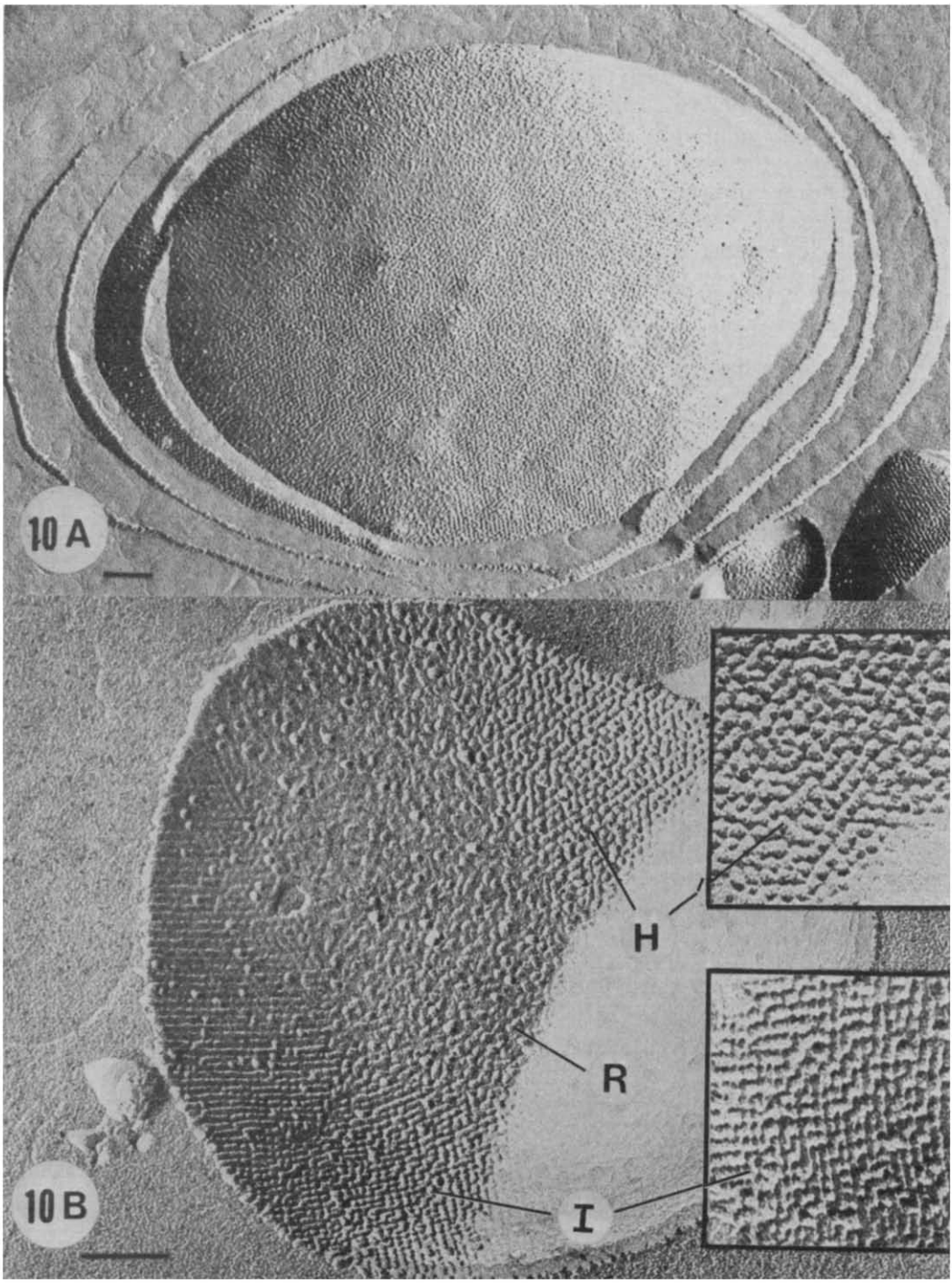


Fig. 10. Electron micrograph of freeze-fractured LHC/phosphatidylcholine vesicles. (A) Preparations were dialyzed vs. 1 mM Tricine/NaOH, 1 mM NaCl prior to freeze-fracture. (B) Preparations were trypsin treated and incubated in the presence of 5 mM $MgCl_2$ prior to freeze fracture. Particle packing was hexagonal (H), random (R), or isometric (I). Bars equal 0.1 μm.

of either unilamellar or multilamellar vesicles (depending on details of LHC/lipid incubation procedures). Unilamellar vesicles (prepared as described by Brunner et al. [22]) with incorporated LHC were uniformly dispersed in 'low-salt' solutions (Fig. 9A). The protein complexes were visible in the bilayer as individual, randomly dispersed particles or as aggregates of crystalline subunits. Upon addition of 5 mM $MgCl_2$, these small vesicles aggregated (Fig. 9B). This densely packed mass of vesicles demonstrates the ability of LHC to mediate vesicle-vesicle adhesion, but the poor fracture pattern through the aggregated material did not facilitate analysis of subunit organization. We have therefore prepared vesicles by sonication of phosphatidylcholine for 30 s at

Fig. 11. Electron micrograph of freeze-fracture of LHC incorporated in phosphatidylcholine vesicles after addition of 5 mM $MgCl_2$. Bar equals 0.1 μm.

4° C, followed by LHC insertion as described above. These preparations are shown in Fig. 10A.

The sonicated LHC/phosphatidylcholine vesicles were found to be a mixed population of single or multilamellar vesicles. The LHC subunits in these vesicles were either dispersed in a monomeric random pattern or packed in hexagonal lattices. Average particle diameter was 80 ± 10 Å.

Trypsin treatment of LHC/phosphatidylcholine vesicles caused a decrease in apparent molecular weight of the LHC polypeptides (as was shown in Fig. 1). Freeze-fracture examination of these modified vesicles showed no change in particle size; there was an alteration in orientation of a portion of the particles, however. This was characterized by the appearance of isometric lattices (particles packed with axis at 90° angles), in addition to the randomly organized or hexagonally packed lattices also seen before trypsin treatment (Fig. 10B).

When 5 mM $MgCl_2$ was added to the large LHC/phosphatidylcholine vesicles, numerous regions of aggregated membranes with extensive areas of planar interaction were clearly evident in the freeze-fracture preparations (Fig. 11). In all regions where the appression occurred, particles of LHC were tightly packed. Regions of smooth lipid membrane, uninterrupted by LHC particles, were occasionally observed in these preparations; these regions were non-appressed with other membranes. A change in particle organization within the membranes was observed to occur concomitantly with membrane appression. Most particles tended to line up end-to-end in parallel rows with no obvious second degree of symmetry. Occasionally, arrays of particles with apparent hexagonal symmetry (axes intersecting at 60° angles) were observed. Less frequently, crystalline aggregates with axes of symmetry lying at 72° to one another were observed. Very few regions of random particle organization were observed in the cation-aggregated preparations.

When trypsin-treated LHC/phosphatidylcholine vesicles were incubated in the presence of $MgCl_2$, no membrane appression (vesicle aggregation) or particle rearrangement occurred. The trypsin treatment had, therefore, eliminated the ability of the particles to undergo cation-mediated adhesion across membranes or to alter their arrangement within individual lipid membranes.

Discussion

A summary of our interpretations of the isolation, characterization, and structural organization of the LHC in these studies is presented in Fig. 12.

In this study we have utilized a light-harvesting pigment-protein which was isolated from chloroplast membranes via mild detergent fractionation techniques to retain native, in vivo characteristics. When precipitated, washed, and dialyzed, the LHC is observed by structural studies to consist of structural units averaging 80 ± 10 Å in diameter. These units interact randomly to form planar sheets, indicating that all particles retain a region of high hydrophobicity (in a ring about each particle) such that edge-to-edge hydrophobic interactions are generated. Presumably this hydrophobic region would be that portion of the total complex which resided in the lipid bilayer in the native membrane; this could consist of hydrophobic side chains of the polypeptides, tightly bound

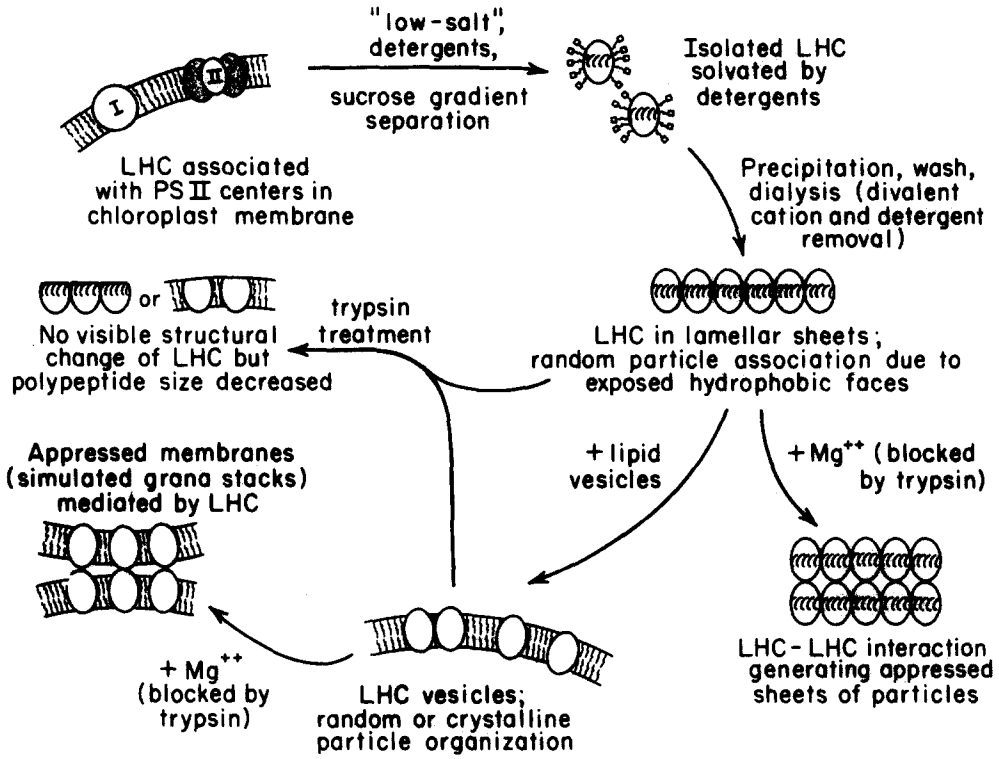


Fig. 12. Diagrammatic representation of our interpretations concerning the isolation, characterization and structural organization of the LHC.

lipids, or phytol chains of the chlorophylls. This interpretation implies that the LHC subunit spans the lipid bilayer in the chloroplast lamellae. This idea is consistent with the following published data. Deep-etch analysis of the inner surface of the thylakoid membrane has revealed the presence of the large freeze-fracture subunit extending into the lumen of the 'chloroplast sac' [23]. The large freeze-fracture particle has been shown to contain the light-harvesting complex [21,24], indicating that the LHC does extend into the inner space bounded by the chloroplast thylakoid. Recent studies using the proteolytic enzyme trypsin [10] and antibodies directed against the LHC [19] have provided evidence that the LHC is also surface exposed in chloroplast thylakoids.

The LHC in the 'low-salt', detergent-depleted form exists as non-appressed particulate sheets. This suggests that electrostatic repulsion may exist between adjacent particle sheets. A similar situation may be present in chloroplast membranes in 'low-salt' solutions and may be involved in potentiating the unstacking of chloroplasts under these conditions [25].

The addition of cations to isolated LHC allows the pigment-protein to cross-link with itself to form appressed lamellar sheets in which the particles arrange in crystalline patterns. The action of cations in this process may be to collapse a repulsive field created by negative surface charges localized on the

LHC particle sheets. The reduction of this repulsive shield may allow expression of a specific adhesive region on the LHC particles. The nature of this specific short-range interaction component which would stabilize membrane association is still unknown; we do note, however, that removal of a 2 kilodalton polypeptide fragment from the surface of the particles by trypsin blocks the short-range interaction process (controlling membrane stacking) in both our isolated particle preparations and in previously analyzed intact chloroplast membranes [9,10].

One of the goals of the current research was to simulate grana stacking in a model membrane system in which the only cation-mediated events could be expressed via a single, defined chloroplast-derived component. As was shown in the comparison of Fig. 2A vs. 2D and Figs. 10A vs. 11, addition of $MgCl_2$ to LHC in uncharged lipid vesicles results in the appearance of large areas of membrane-membrane contact. This membrane appression appears to mimic that occurring during the formation of grana stacks. Under the existing circumstances, the appression can only be mediated by LHC, since the uncharged lipid vesicles by themselves showed no alteration upon addition of $MgCl_2$. In addition, trypsin treatment of the LHC blocked the cation-mediated aggregation phenomenon. We conclude that the surface-exposed segment of the LHC which is removed by trypsin is responsible for the short-range interactions generating membrane appression in the model system. The cation concentration requirement needed to elicit membrane stacking in the LHC/phosphatidylcholine vesicle experiments (Fig. 8) is very similar to that observed with intact chloroplast membranes. The higher concentration requirement for monovalent cations needed to elicit the same aggregation is also consistent with studies on intact membranes [3,12].

It was observed in this study that cation-induced lamellar appression occurs concomitantly with the reorganization of the particles within the membranes. In the 'low-salt' vesicles, LHC existed either in random or hexagonal patterns of crystallization. Upon cation addition, almost all of the random patterns were lost and particles were more frequently seen in linear arrays, with regions of predominantly hexagonal packing. Cation addition to trypsin-treated vesicles (which did not appress) did not result in changes in particle organization although the trypsin treatment itself caused formation of isometric arrays. These data demonstrate that changes at the surface-exposed portions of the LHC or interactions between these components can influence the orientation of these particles within the lipid phase of the membrane. Since trypsin treatment of the LHC in vesicles did not release any free chlorophyll, we conclude that the chlorophyll contained in these particles is probably localized in the more hydrophobic region of the particle which is inaccessible to trypsin digestion. The data therefore suggest that cation-mediated alterations in the surface-exposed segments of the LHC may alter the orientation of the light-harvesting pigments with respect to adjacent particles. These changes in light-harvesting particle-particle orientation could dramatically alter interunit (LHC → PS I, PS II) energy transfer and account for cation-mediated excitation energy redistribution [14,15].

In this report it was noted that a new spectral species, C-686—F-695, was formed concomitant with the transition from detergent-solvated LHC to

particle sheets. Detergent solvation of LHC was reversible and di not release free chlorophyll. Solvated LHC as compared to free chlorophyll, exhibited a red shift in fluorescence emission maximum (680 nm compared to 670—675 nm for free chlorophyll). This is probably due to Chl-protein ligation consistent with observations made in vivo [26]. The formation of an additional red-shifted spectral species, C-686—F-695, concomitant with the transition from solvated LHC to LHC particle sheets, suggests that new pigment-protein or pigment-protein interactions within or between LHC particles, have occurred.

In vivo F-695 has been designated as arising from PS II traps [27] or from an antennae near the PS II trap [28]. Rijgersberg et al. [29] have recently reported that the yield of F-695 increases dramatically over the temperature range 100—40 K, while PS II photochemistry remained constant. This observation suggests that F-695 arises from antennae species which exhibits temperature-dependent energy transfer to the PS II reaction center. Our results are consistent with this interpretation and we suggest that C-686 found in the LHC pigment bed many contribute to F-695 in vivo.

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