

THE LIGHT-HARVESTING CHLOROPHYLL- PROTEIN COMPLEX OF PHOTOSYSTEM II

Its Location in the Photosynthetic Membrane

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ABSTRACT

We have investigated the structure of the photosynthetic membrane in a mutant of barley known to lack a chlorophyll-binding protein. This protein is thought to channel excitation energy to photosystem II, and is known as the "light-harvesting chlorophyll-protein complex." Extensive stacking of thylakoids into grana occurs in both mutant and wild-type chloroplasts. Examination of membrane internal structure by freeze-fracturing indicates that only slight differences exist between the fracture faces of mutant and wild-type membranes. These differences are slight reductions in the size of particles visible on the EFs fracture face, and in the number of particles seen on the PFs fracture face. No differences can be detected between mutant and wild-type on the etched outer surface of the membrane. In contrast, tetrameric particles visible on the etched inner surface of wild-type thylakoids are extremely difficult to recognize on similar surfaces of the mutant. These particles can be recognized on inner surfaces of the mutant membranes when they are organized into regular lattices, but these lattices show a much closer particle-to-particle spacing than similar lattices in wild-type membranes.

Although several interpretations of these data are possible, these observations are consistent with the proposal that the light-harvesting chlorophyll-protein complex of photosystem II is bound to the tetramer (which is visible on the EFs face as a single particle) near the inner surface of the membrane. The large tetramer, which other studies have shown to span the thylakoid membrane, may represent an assembly of protein, lipid, and pigment comprising all the elements of the photosystem II reaction. A scheme is presented which illustrates one possibility for the integration of the light reaction across the photosynthetic membrane.

The light reaction of photosynthesis is localized within the thylakoid membranes found in higher plants and green algae. The structure of these membranes is exceedingly complex, but now seems to be understood in general terms. At least two types of particles exist within the membrane, and can be visualized by the freeze-fracture tech-

nique (10, 14, 22). The larger of these particles is found principally in stacked (grana) regions of the chloroplast (14, 33, footnote 1), spans the thyla-

¹ Staehelin, L. A. 1976. Reversible particle movements associated with unstacking and restacking of chloroplast membranes in vitro. *J. Cell Biol.* **71**:136-158.

koid membrane (20), is visible at both surfaces of the membrane (20), and may make contact with small particles on adjacent membranes in stacked regions (31). In addition to these integral membrane structures, particles are also found on the outer surface of the thylakoid which can be removed by washing in appropriate buffers. Several groups have identified these particles as individual molecules of the coupling factor (an enzyme involved in the synthesis of ATP during electron transport) (7, 11, 23, 26) and possibly of carboxydismutase (the primary enzyme in the cycle of carbon fixation) (23). These particles seem to be found only on membrane surfaces in unstacked (stromal) regions of the chloroplast (23).

The structures revealed within the membrane by freeze-fracturing have yet to be identified. We report in this paper a study on mutant chloroplasts lacking a component of the photosynthetic apparatus. This work was undertaken with the hope of detecting structural alterations in the mutant which would allow us to determine the actual location of the missing component in wild-type chloroplasts.

A pale green mutant of barley was reported in 1950 by Highkin (16), and was subsequently shown to be completely devoid of chlorophyll *b* (17). In later studies, this mutant was also found to lack a chlorophyll-binding protein (molecular weight approximately 35,000 daltons) associated with photosystem II (35). Although this protein is distinct from the actual reaction center of photosystem II (since the mutant lacking it possesses high rates of noncyclic electron transport and oxygen evolution [8]), it seems to follow the spatial distribution of photosystem II in the membrane (1), and may have as its primary function the channeling of energy to the photosystem II reaction center (33). Thornber (33) has termed this component the "light-harvesting chlorophyll *a/b* protein." In this paper we explore structural alterations in Highkin's barley mutant (16) lacking the protein.

MATERIALS AND METHODS

Seeds of the chlorophyll *b*-lacking barley mutant were kindly provided by Dr. Harry Highkin of California State University. Wild-type seed was obtained from a local supplier. The seeds were planted in vermiculite, watered daily with 0.25 strength Hoagland's solution, and grown under a 16-h day, 8-h night regime at 20°C in a growth chamber. Light was supplied by a mixture of fluorescent and incandescent bulbs. Light intensity at the level of the

seedlings was 13,000 lx. Plants were harvested for experimentation after 3 wk growth.

Chloroplasts were isolated by differential centrifugation as described elsewhere for spinach leaves (23). Leaves were prepared for thin-sectioning as described elsewhere (21).

Isolated chloroplasts were prepared for freeze-fracturing by infiltration with glycerol to a final concentration of 30% vol/vol. Isolated chloroplasts were prepared for deep-etching by washing in a dilute buffer as described elsewhere (23). Samples were frozen in liquid Freon and stored in liquid nitrogen. Replicas were prepared on a Balzers freeze-etching device (Balzers High Vacuum Corp., Santa Ana, Calif.) according to the method of Moor and Mühlethaler (24). Successful replicas were cleaned in bleach and examined in a Philips 300 electron microscope operated at 80 kV.

Measurements of particle sizes and spacings were made on micrographs enlarged to $\times 200,000$ and examined with a $\times 7$ ocular fitted to a micrometer scale. Gel electrophoresis was performed according to methods described elsewhere (4), except that gels of 10% polyacrylamide were used rather than gradient gels.

RESULTS

Thin-Sectioning

Examples of wild-type and mutant chloroplasts are shown in Figs. 1 and 2. An earlier report on chloroplast structure in this mutant emphasized the point that membrane stacking was reduced in the mutant (13). We have not completed a careful analysis of the extent of membrane contact in the mutant, but it would clearly be a mistake to conclude that the loss of the chlorophyll-protein complex causes a dramatic reduction in stacking. The membrane system in mutant chloroplasts does seem somewhat less organized in general, but the essential feature of higher plant chloroplasts, a thylakoid system organized into discrete grana connected by single stroma membranes, is present in both mutant and wild-type chloroplasts.

Polyacrylamide Gel Electrophoresis

If purified thylakoid membranes are subjected to polyacrylamide gel electrophoresis under certain conditions, chlorophyll-containing proteins can be separated on the gel still retaining at least part of their bound pigment (4). This procedure allows chlorophyll-binding proteins to be identified on the gel before staining and then compared with the full complement of membrane proteins made visible by the stain. Fig. 3 shows the appearance of SDS-polyacrylamide gels of wild-type and

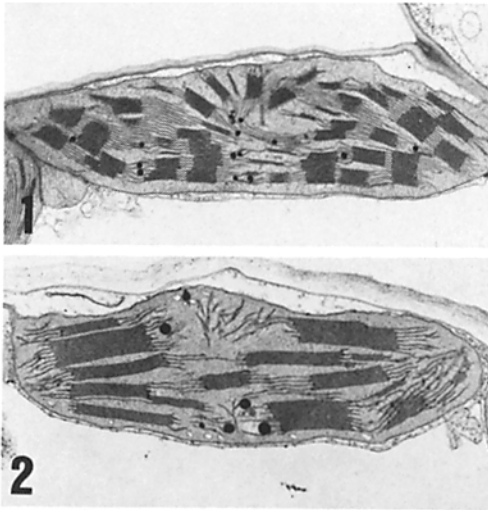


FIGURE 1 Barley chloroplast in thin section. This plastid is representative of wild-type chloroplasts. Numerous grana stacks containing as many as 25 thylakoids are visible. Several dark-black lipid droplets are visible in the chloroplast. Magnification: 8,000.

FIGURE 2 Thin section of chloroplast from the chlorophyll *b*-lacking mutant of barley. Thylakoid membranes are still organized into distinct grana with interconnecting stroma (unstacked) membranes, but the membrane system is less organized than in the wild-type. Nevertheless, the mutant is still quite clearly capable of forming extensive stacked regions. Magnification: 12,000.

mutant barley chloroplasts before and after staining. In the unstained gels, two main bands are visible in the mutant, and three in the wild-type. Both chloroplasts contain a high molecular weight component associated with photosystem I (33). Free pigment runs at the leading edge of each gel. Each gel also contains a very faint band of intermediate molecular weight. Wild-type membranes contain, in addition to these, an intense green band of low molecular weight just above the free pigment zone. This band has been identified as the light-harvesting chlorophyll-protein complex of photosystem II (33). This band is entirely absent in the mutant chloroplast membrane.

Staining makes visible the full complement of membrane proteins. As can be seen in Fig. 3, the protein band corresponding to the light-harvesting chlorophyll-protein complex of photosystem II is completely missing, indicating that the loss of this band in the unstained gel was due to a complete absence of protein at this point in the gel, not just to a loss of pigment from the protein moiety of the

complex. Similar results with a variety of different gel systems have been reported by a number of workers (2, 3, 12, 15, 35).

Freeze-Etching

The missing protein band accounts for a large percentage of total membrane protein, and its loss might be expected to cause a dramatic alteration in membrane substructure. However, as shown in Figs. 4 and 5, surprisingly few changes are revealed by the freeze-fracturing technique. Four

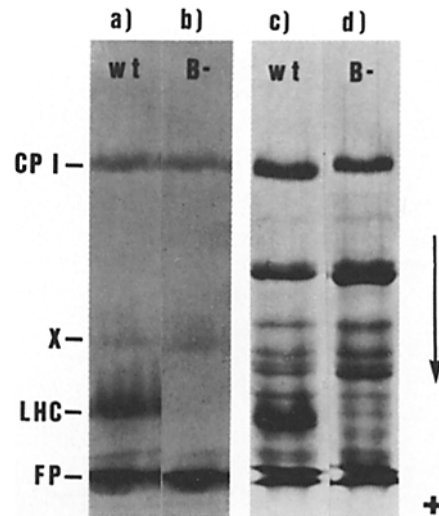


FIGURE 3 (a) Unstained SDS-polyacrylamide gel prepared as described in text. The conditions under which this gel of wild-type chloroplast membrane proteins was run do not dissociate all pigment from the chlorophyll-binding proteins, and they are visible before staining as green bands. Labeled are CP 1—the chlorophyll-protein complex of photosystem I; X—a chlorophyll-protein complex of intermediate molecular weight and uncertain origin; LHC—the light-harvesting chlorophyll-protein complex of photosystem II; FP—free pigment. (b) Unstained slab gel prepared from chloroplast membranes of the barley mutant. The light-harvesting chlorophyll-protein complex of photosystem II is missing. Other bands appear similar to the wild-type. (c) A gel of wild-type membranes from the same slab as Fig. 3a, now stained to reveal nonpigmented proteins. Note especially the large protein band corresponding to the light-harvesting complex visible in Fig. 3a. (d) A gel of mutant chloroplast membranes, from the same slab as Fig. 3b, now stained. The protein pattern is essentially the same as Fig. 3c, except that the large band identified as the light-harvesting chlorophyll-protein complex is missing. This indicates that the mutant is devoid of both the pigment and protein moieties of the complex.

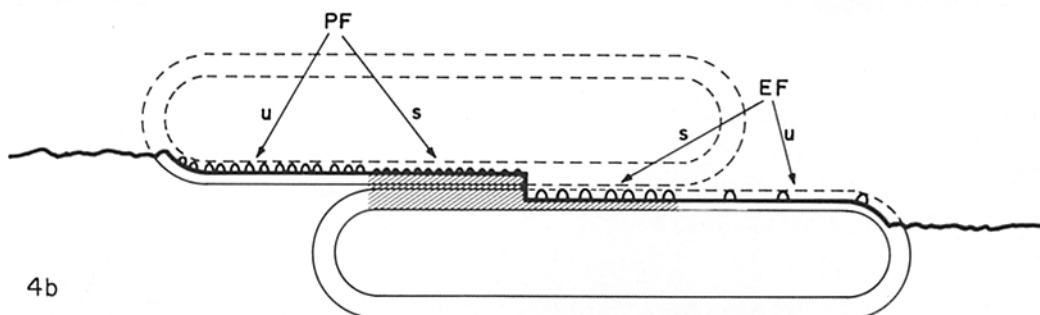
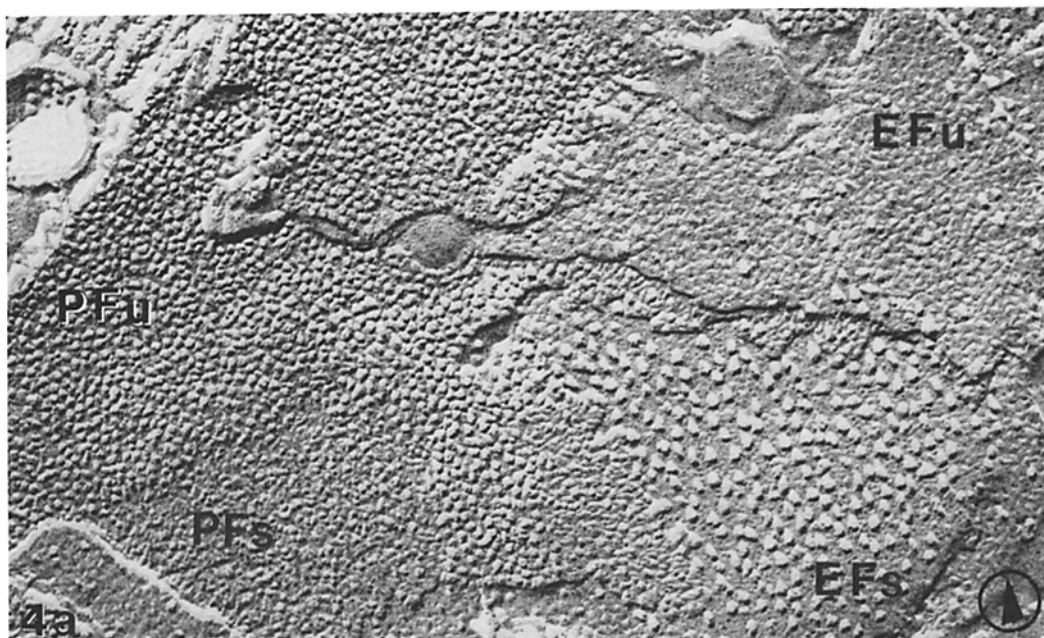


FIGURE 4 (a) Freeze-fracture replica of isolated wild-type thylakoids. Four faces are clearly present, and are labeled according to the system of Staehelin (see footnote 1). These faces have been described in detail for other species, and are typical of higher plant photosynthetic membranes. Magnification: 100,000. (b) Diagram illustrating the derivation of each of the four faces illustrated in Figs. 4a and 5. The stacked region in the center of the diagram is shaded. Compare this diagram with Figs. 4-7 in order to determine the location of various particles within the photosynthetic membrane.

distinct fracture faces are observed in wild-type chloroplasts: two from the splitting of membranes in stacked regions, and two from the splitting of unstacked membranes (14). These faces have been well characterized by other workers (22, 25; footnote 1) and are labeled according to the nomenclature of Staehelin (see footnote 1).

Mutant chloroplasts display essentially the same four faces, as shown in Fig. 5, with some slight but significant differences. Although the faces derived from splitting membranes in unstacked regions are quite similar to those of wild-type membranes

(PFu and EFu), the particles on the EFs fracture face seem slightly smaller than those on the corresponding wild-type face. Also, there seems to be a definite reduction in the number of particles visible on the PFs fracture face of the mutant compared to the wild-type. Both the EFs and PFs faces are formed from the splitting of membranes in grana stacks (see Fig. 4b). Higher-magnification views of these two faces are shown in Figs. 6 and 7. Note once again that the EFs particles seem a bit smaller in the mutant, and that the number of particles visible on the PFs fracture face is re-

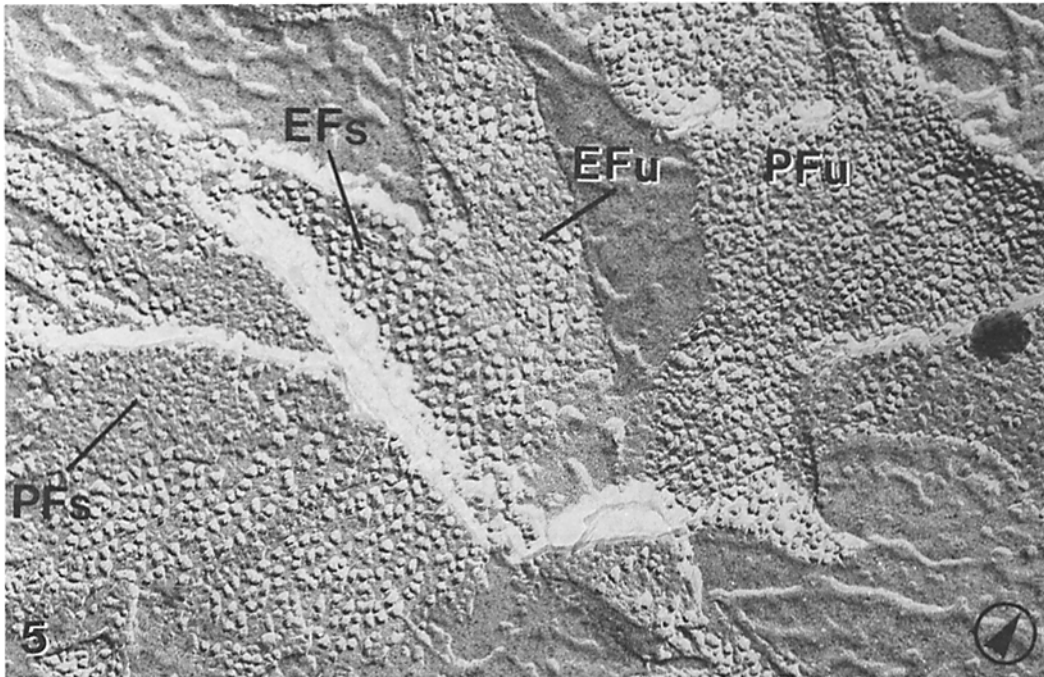


FIGURE 5 Freeze-fracture replica of isolated chloroplasts from the barley mutant. Four faces (see Fig. 4a, b) are marked. Two differences are apparent in the faces derived from membrane splitting in stacked regions: particles on the EFs fracture face seem somewhat smaller than in wild-type membranes, and there seem to be fewer particles present on the PFs face. Magnification: 100,000.

duced. Although Henriques and Park (15) recently claimed that no differences were evident in freeze-fractured preparations of this mutant compared to the wild-type, we do not think that our results are necessarily in conflict with theirs. The reduction in particle size is slight (see Fig. 8) and could be overlooked, and the figures presented by Henriques and Park (15) do not clearly show each of the four faces illustrated here. It is therefore not possible to determine from their micrographs whether a change was present in the PFs fracture faces they examined.

Because the freeze-fracture technique splits biological membranes along a roughly central plane (9), thereby limiting information to whatever structures are present at this level in the thylakoid, we also examined the actual surfaces of the membrane in an effort to determine whether the absence of the chlorophyll-binding protein might cause more noticeable changes at or near the true surfaces of the membrane.

The outer surfaces of higher plant thylakoids have been analyzed in detail elsewhere (23), and the outer surfaces of both mutant and wild-type

barley thylakoids are quite similar to those reported for spinach (23). In comparison to spinach thylakoids, fewer of the large particles identified as coupling factor molecules were present on the membranes we examined. This could be the result of interspecies differences or merely of the loss of coupling factor molecules during membrane isolation. No differences were observed (Figs. 9, 10) between mutant and wild-type thylakoids on their outer surfaces.

In contrast, the inner surfaces of mutant and wild-type thylakoid membranes show dramatic differences. The inner surface of the photosynthetic membrane of wild-type barley is shown in Fig. 11. The inner surface is studied with tetrameric particles measuring approximately $140 \times 190 \text{ \AA}$. These particles are concentrated into roughly circular regions which correspond to individual grana stacks (32; footnote 1). The inner surface of the mutant thylakoid shows a much less organized structure (Fig. 12). Large numbers of small particles are present, although at higher magnifications (*inset*, Fig. 12) the particles can, in some cases, be seen to display what may be a

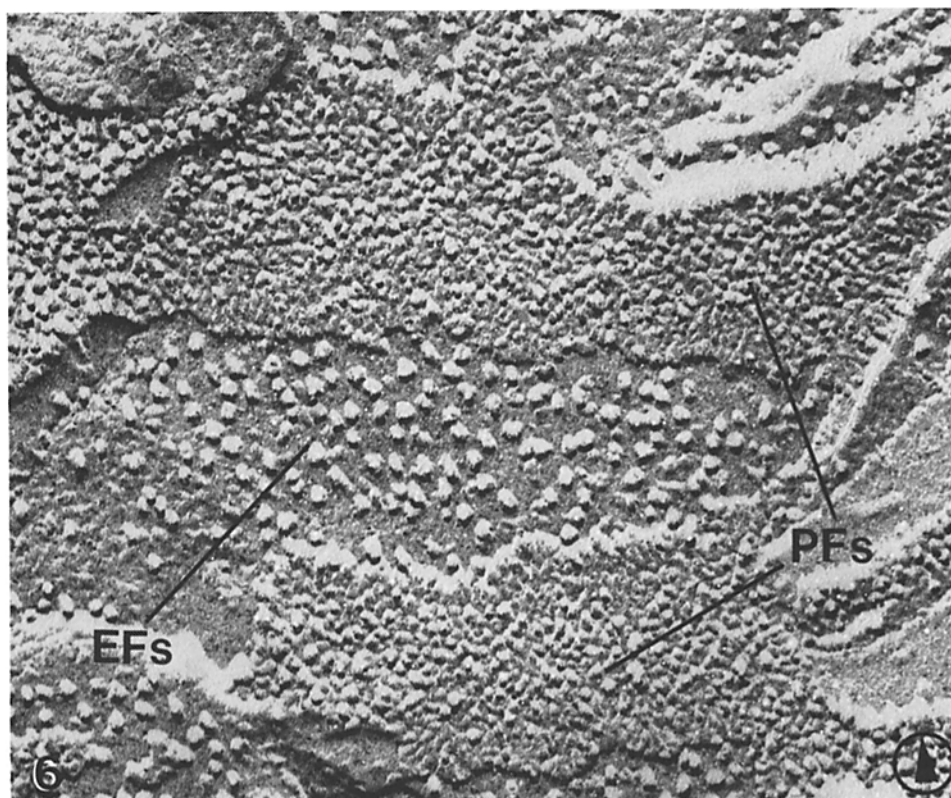


FIGURE 6 High magnification of a portion of wild-type membranes freeze-fractured in a stacked (grana) region in the center of the diagram is shaded. Compare this diagram with Figs. 4-7 in order to determine the location of various particles within the photosynthetic membrane. Magnification: 150,000.

tetrameric substructure. Nevertheless, this substructure is much less apparent and less clearly defined than that of the wild-type.

Many workers have reported that particles on this surface are occasionally organized into regular lattices (20, 27, 29). These configurations do not occur in all membrane preparations, and we are not certain of the factors governing their appearance. Nevertheless, we have observed them in wild-type membranes, as shown in Figs. 13 and 14. These lattices should not be taken as representative of normal membrane structure, although they do provide a convenient means of measuring minimum particle spacings. The lattices shown in Figs. 13 and 14 are typical of wild-type membranes, and display spacings of $180 \times 225 \text{ \AA}$ and $180 \times 240 \text{ \AA}$, respectively.

Lattices are also observed in some preparations of mutant chloroplasts, as shown in Figs. 15 and 16. Although these lattices have the same basic organization as those in wild-type membranes, the

four subunits of each tetramer are so small as to be at the limit of resolution for the freeze-etch replication process. Spacings in these lattices measure $90 \times 140 \text{ \AA}$ (Fig. 15) and $105 \times 150 \text{ \AA}$ (Fig. 16). Lattices such as these in the mutant thylakoid membranes make it clear that particles with tetrameric substructure are still present on the inner surface, but these particles are smaller than wild-type tetramers, pack more closely, and do not protrude as far from the membrane surface (accounting for our difficulty in visualizing them in shadowing replicas).

In summary, the changes brought about by the mutation seem to be: (a) a reduction in size of the EFs particle; (b) the presence of fewer particles on the PFs face; and (c) a large decrease in the size and visibility of tetrameric particles on the inner surface of the thylakoid sac.

DISCUSSION

As other workers have shown (12, 35), the chloro-

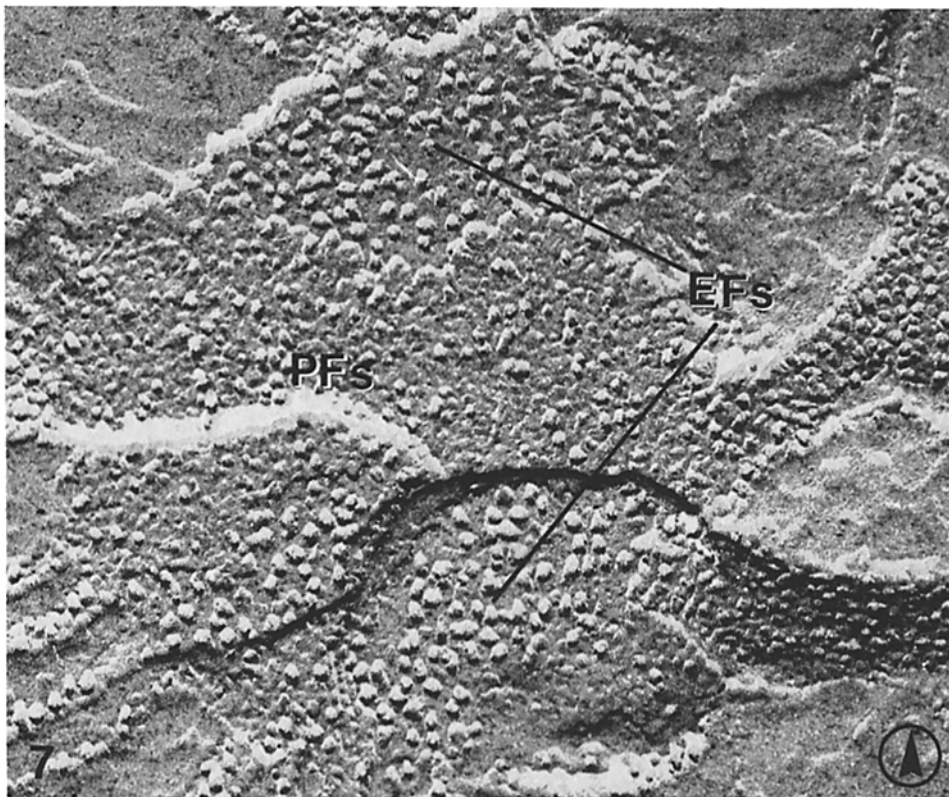


FIGURE 7 High magnification of a grana from the mutant chloroplast. The same two faces as in Fig. 6 are visible, and the reductions in particle number on the PFs face and in particle size on the EFs face are clearly apparent. Magnification: 150,000.

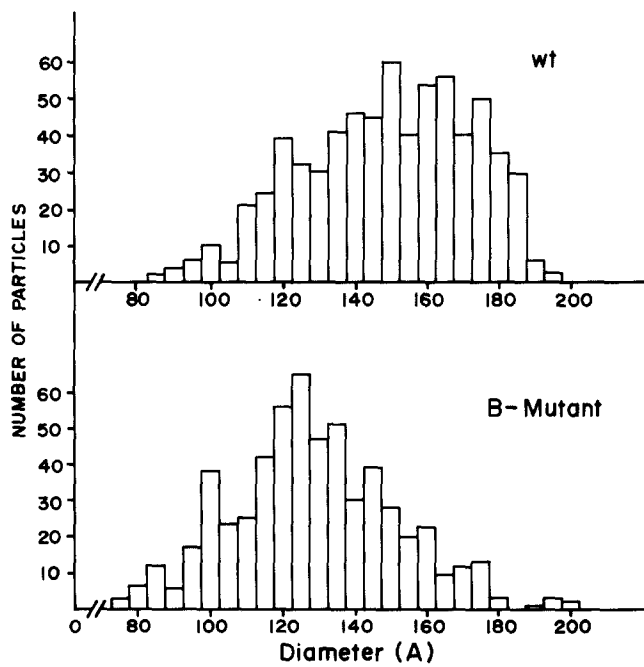


FIGURE 8 Histograms of particle sizes in the EFs faces of chlorophyll *b*-lacking mutant (*B*-) and wild-type (*WT*) chloroplasts.

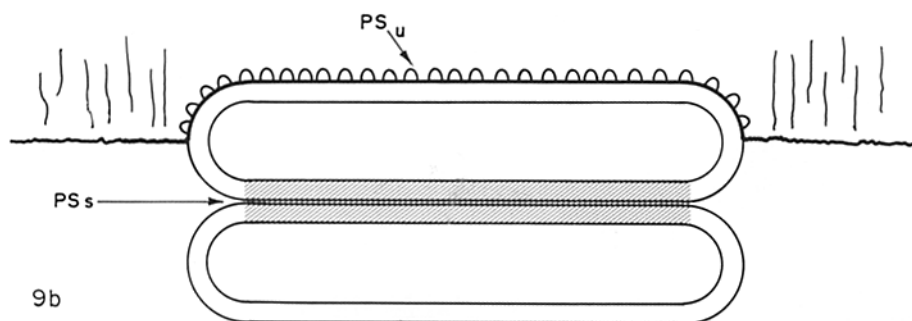
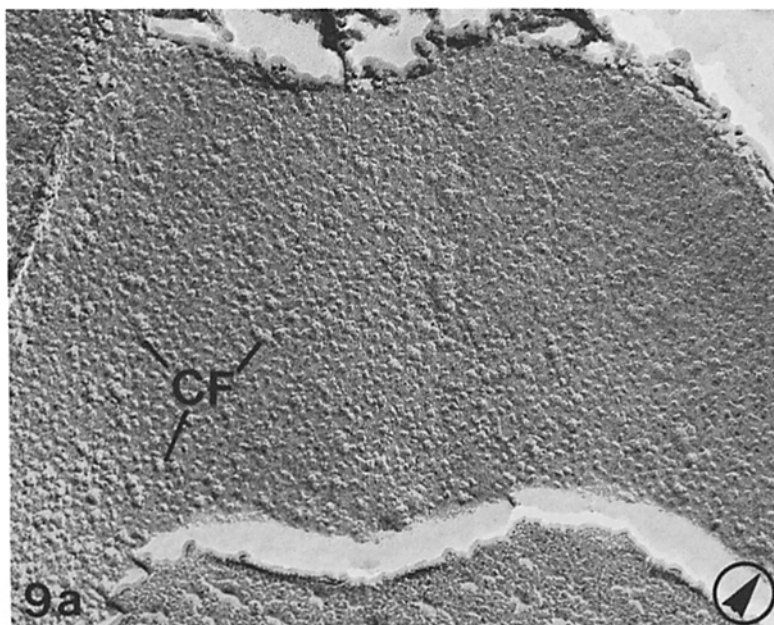


FIGURE 9 (a) Outer surface of a wild-type thylakoid. This surface is revealed by the etching away of frozen dilute buffer from above the membrane. The surface is covered with small particles and larger ones (CF) which in other species have been identified as coupling factor molecules. Magnification: 100,000. (b) Diagram illustrating how the actual outer surface of the thylakoid may be observed by deep-etching. The fracture occurs at a level above the frozen membranes, and it is the actual sublimation of buffer from the frozen surface which exposes the membrane surface. Note that the outer surface of the membrane in stacked regions (PSs) cannot be observed due to the close apposition of the adjacent membrane.

phyll *b*-lacking mutant of barley does not contain a low molecular weight chlorophyll-protein complex. This mutant clearly lacks both the pigment and protein moieties of the complex, since no additional protein bands appear in mutant gel profiles (see Fig. 3).

Three basic changes in membrane structure are present in the mutant: (a) a decrease in the diameter of the large EFs particle; (b) a reduction in the number of small particles visible on the PFs fracture face; (c) a decrease in the size and visibility of

the ESs tetramer which is so extensive that we can present convincing micrographs which show the continued presence of the particles only when they are organized into repeating lattices.

In trying to formulate a reasonable scheme relating these structural changes to biochemical alterations in the mutant, we face a difficulty which in one form or another is common to most current work on membrane structure. Specifically, the level at which we are able to probe the structure of the photosynthetic membrane (by use of the

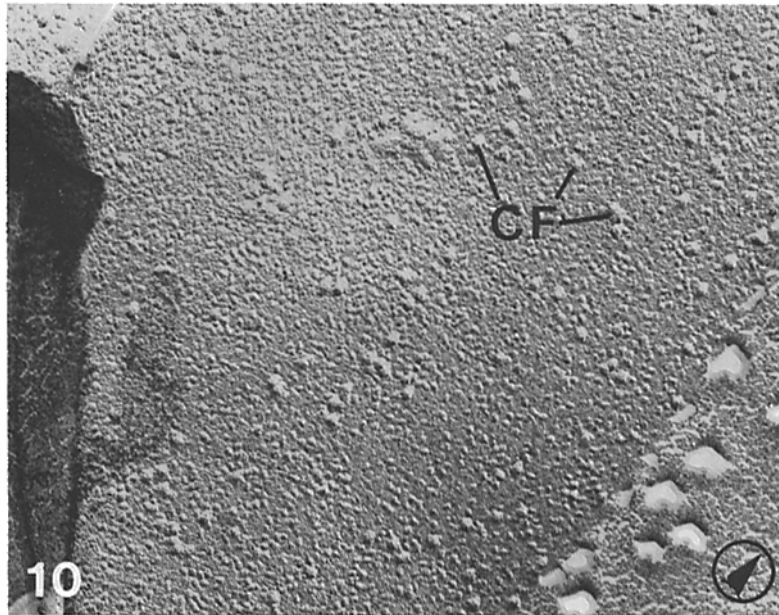


FIGURE 10 Outer surface of a mutant thylakoid revealed by deep-etching (see Fig. 9*a, b*). Coupling factor molecules (*CF*) are marked. No differences are apparent between the outer surfaces of the mutant thylakoid and the wild-type. Magnification: 100,000.

freeze-etching technique, for example) falls far short of the level at which we are able to describe its energetic and biochemical properties. As many as 60 polypeptides make up the photosynthetic membrane (1, 4, 5, 15). In contrast, a current estimate of the number of specific subunits in this membrane shows that only four or five distinct particles can be resolved within and on the thylakoid (1, 20, 23, 31).

This is not to suggest that large numbers of particles wait as yet undetected within the photosynthetic membrane, but only to emphasize the current conception (1) that many distinct polypeptides, pigment molecules, and other components may be bound up in such a way that large structures corresponding to the particles that we are able to observe in the electron microscope are formed. This means that at best, given the list of structural changes we have observed in association with this mutation, we can only hope to implicate one or more particles as containing the light-harvesting complex (in wild-type membranes), and then to make some informed guesses as to what implications our findings have for the structural organization of the light reaction of photosynthesis.

Two recent findings concerning thylakoid structure should be pointed out which bear on these

results: Miller (20) has shown that both the EFs particle and the ESs (inner surface) tetramer are formed by a single structure which spans the thylakoid membrane; and Staehelin (31) has shown that this membrane-spanning tetramer makes contact with the small particles visible on the PFs fracture face in stacked regions.

These findings raise the possibility that each of the three changes in membrane structure may arise from the absence of the light-harvesting complex if that complex were associated with the tetramer (in the wild-type) in such a way that its absence caused: (*a*) changes in the size and shape of the membrane-spanning tetramer visible in both EFs and ESs views of the membrane; and (*b*) a reduction in ability of the tetramer to make contact with the small particles seen on the PFs face, so that fewer of them would be bound in stacked regions.

There are several ways in which this might take place. The complex may be positioned towards the inner half of the membrane in contact with the membrane-spanning tetramer. This would account for the fact that only slight changes are observed on the EFs face in the mutant (Figs. 4–8). If the complex is near the inner surface of the thylakoid and is associated with the tetramer (or actually forms part of it), then its absence could obviously

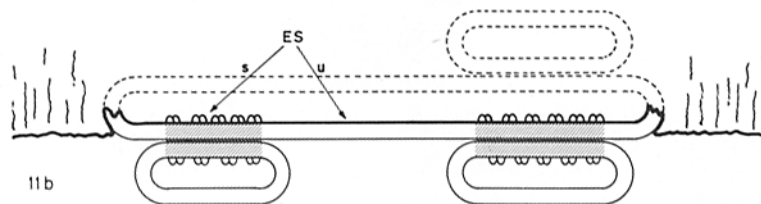
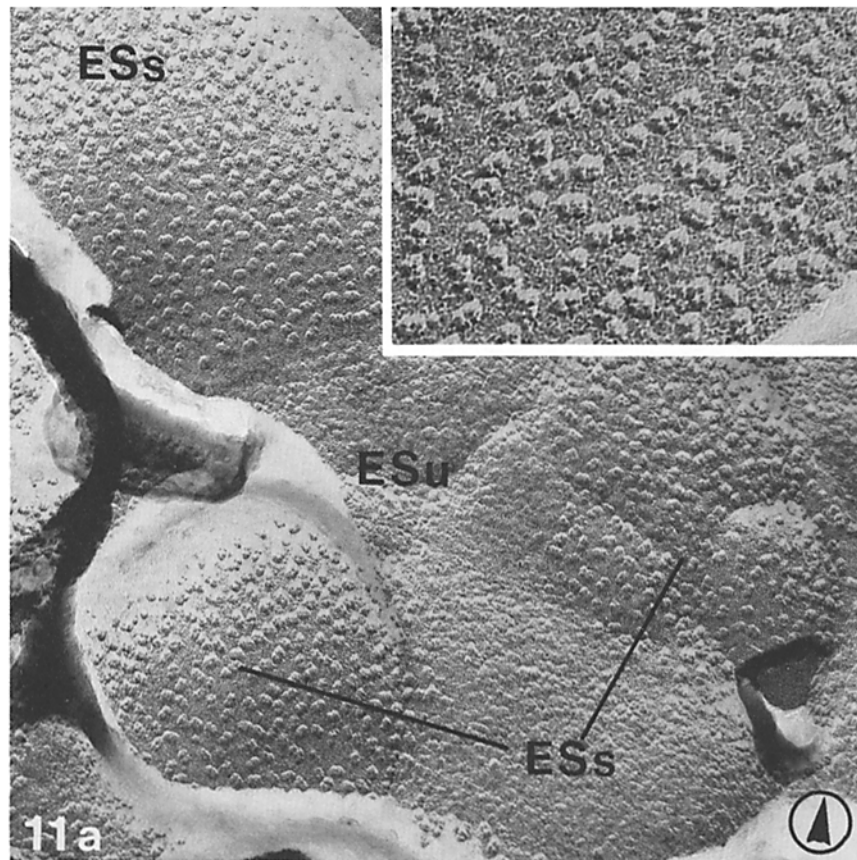


FIGURE 11 (a) Inner surface of the thylakoid membrane of wild-type barley chloroplasts. Both stacked (*ESs*) and unstacked regions are visible (*ESu*). Note that large particles are concentrated in stacked regions, and are virtually absent from unstacked regions. Magnification: 100,000. The inset shows a higher magnification view of such particles in a stacked region. These particles measure approximately $190 \times 130 \text{ \AA}$, and four subunits are clearly visible in most of the particles. Magnification of inset: 200,000. (b) Diagram illustrating how fracturing and deep-etching in sequence can be used to examine the actual inner surface of the thylakoid membrane. The upper portion of the granum is first fractured away (dotted lines represent membranes removed by the fracturing process), and the sublimation of frozen buffer exposes the inner surface of the membrane as shown. Stacked and unstacked regions may both be viewed in this way from the inner surface.

cause the tetramer to appear much smaller on the membrane inner surface. Two possibilities illustrating this point of view are diagrammed in Fig. 17. The work presented here does not allow us to distinguish between these alternatives, although a

recent study by Apel and his associates (5) suggests that the light-harvesting chlorophyll-protein complex in *Acetabularia* is not exposed to the membrane surface, due to its inaccessibility to surface probes.

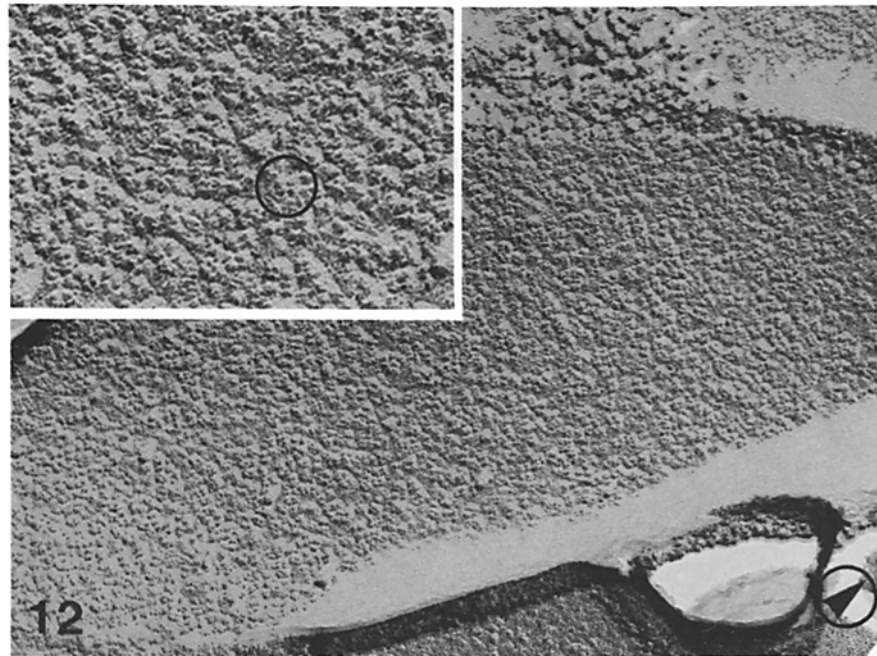
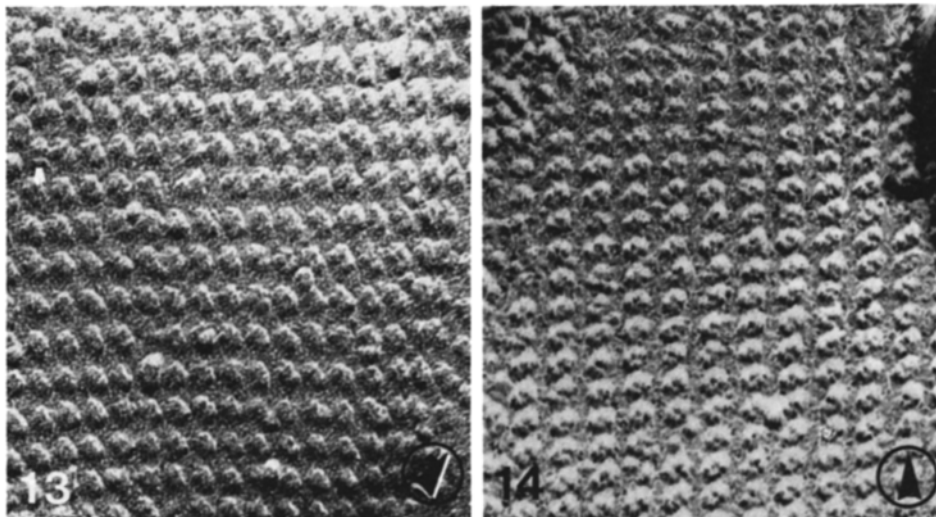
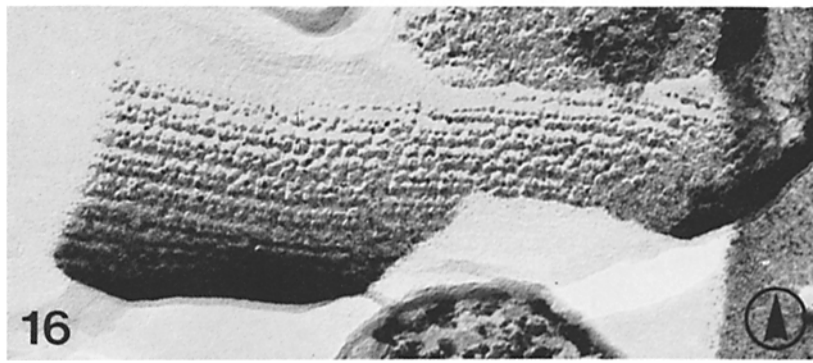
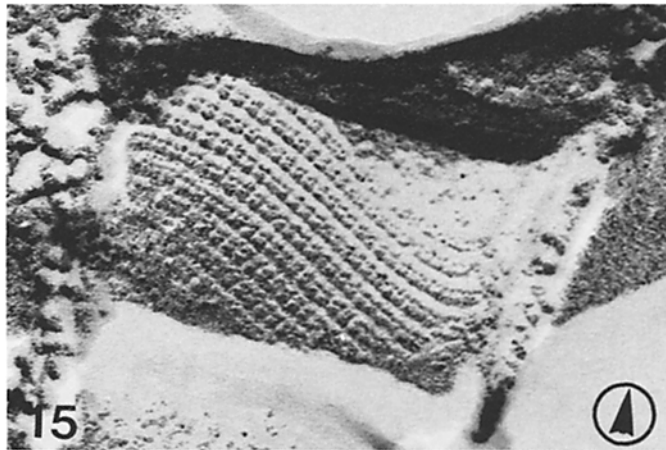


FIGURE 12 Inner surface of a mutant thylakoid membrane. In contrast to the wild-type, particles are not clearly visible on the irregular surface. Possibly because of the difficulty of recognizing individual particles on this surface, we have not been able to delineate individual stacked and unstacked regions. Magnification: 100,000. The inset shows a higher magnification view of the surface, and at least one tetramer similar to the wild-type particles is circled. Attempts to measure the few recognizable tetramers show them to be approximately $90 \times 140 \text{ \AA}$. Magnification of inset: 200,000.



FIGURES 13 and 14 Repeating lattices of tetrameric particles on the inner surfaces of wild-type thylakoids. These lattices are observed in some preparations, and we are not certain of the factors governing their presence or absence. They allow a minimum spacing between particles to be determined. The particle-to-particle spacing in Fig. 13 is $180 \times 225 \text{ \AA}$, and in Fig. 14 it is $180 \times 240 \text{ \AA}$. Magnification: 200,000.



FIGURES 15 and 16 Repeating lattices of particles on inner surfaces of the mutant thylakoids. As with the wild-type, such lattices occur only rarely. Compared with Figs. 13 and 14, the much closer packing of particles in these lattices is striking ($90 \times 140 \text{ \AA}$ for Fig. 15; $105 \times 150 \text{ \AA}$ for Fig. 16). As discussed in the text, our interpretation is that the absence of the light-harvesting complex of photosystem II causes a reduction in the size of the tetramer, allowing it to pack into much smaller lattices. Magnification: 200,000.

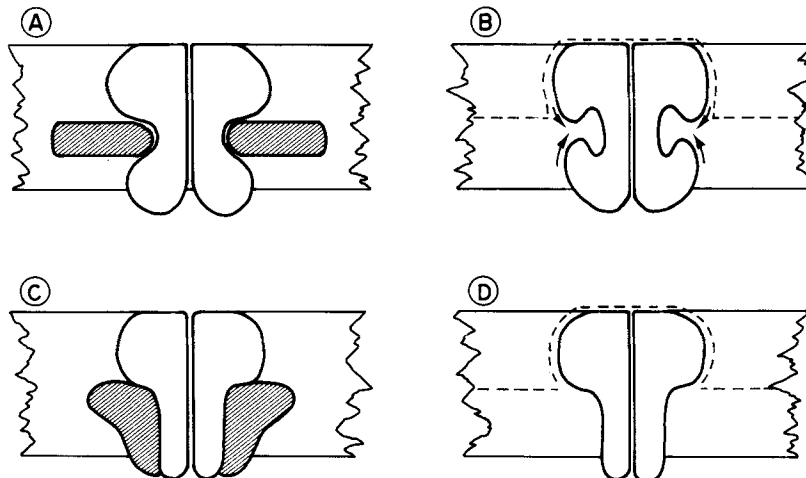


FIGURE 17 Two possibilities for the placement of the light-harvesting complex near the photosystem II tetramer. A, This diagram illustrates how the complex might be placed so that it is not in contact with the inner surface (ES). B, Our conception of how the tetramer in the mutant would be affected by the absence of the complex. A dotted line shows the probable locus of the fracture plane. Arrows illustrate how the absence of the complex might cause less of the particle to protrude from the membrane surfaces. C, An alternative model where the complex is exposed at the inner surface of the membrane. D, How the structure of the mutant might be affected if the tetramer in the wild-type were positioned as in Fig. 17c.

Each of the models in Fig. 17 would also account for the substantial changes seen on the inner surface of the thylakoid (see Figs. 11-16). It is significant, however, that the tetrameric nature of the inner surface particles is still preserved in the mutant, despite the absence of the complex. Clearly, the presence of these unusual structures is not dependent on the inclusion of the light-harvesting complex in the photosynthetic membrane.

The absence of the complex from the tetramer may also account for the reduction in the number of particles visible on the PFs fracture face, since the absence of a component in the interior might cause the tetramer to be less exposed on the outer surface of the membrane. Large-to-small particle contacts might then be less stable, resulting in fewer small particles being present in a stacked region at a particular time.

Although this explanation has some attractive features which we will point out, a number of other possibilities are worth noting. For example, if the light-harvesting complex was bound at or near the outer (PS) region of the tetramer, its absence in the mutant might also diminish its ability to bind the small PFs particle in stacked regions. If the spacings between tetramers in lattice regions were determined at this level, the effect noted in Figs. 13-16 could be produced, and the visibility of the tetramer on the inner (ES) surface might also be affected by an inward collapse of the tetramer to take up the "empty" space vacated by the missing complex.

A third possibility is that the light-harvesting complex might correspond to the PFs particles which seem to be missing in freeze-fracture images of mutant thylakoids (Figs. 4-7). This possibility suggests that the observed interactions between large and small particles in stacked regions (31) may be related to interactions between the light-harvesting complex and the tetramer (photosystem II reaction complex?). Given the overriding problem, namely that we are unable to observe the light-harvesting chlorophyll-protein complex as a discrete structure, other explanations are surely possible, in addition to these three.

In a strict sense, we have presented results which suggest a number of explanations but do not allow us to select any of them in preference to the rest with great certainty. It is important, however, to point out difficulties associated with some of these explanations which may be useful in drawing up a hypothetical scheme for the structural organization of the light reaction.

The main difficulty with the third explanation

(mentioned just above) is that it fails to account in a straightforward way for the dramatic changes seen on the inner surface of the thylakoid. Although it may be suggested that the reductions in tetramer size and spacing occur because of the absence of interactions between large and small particles in the mutant, such interactions cannot occur in unstacked wild-type membranes either, yet no such spacing changes are seen in these. So this explanation is at best strained when it comes to accounting for all our observations. The possibility that the light-harvesting complex may be bound near the outer (PS) surface of the tetramer accounts quite nicely for the changes we have noted in particle spacing on the inner surface, as well as for the reduction in the number of small particles on the PFs fracture face. But if this were indeed the location of the complex, we would expect much larger changes to be evident in fracture (EFs) images of the tetramer. In fact, the changes seen in such images are relatively slight (Figs. 4-8) and fail to support this idea.

The scheme which we believe presents the fewest difficulties is the one which we have discussed in some detail and which is presented in diagrammatic form in Fig. 17. As noted, all of our observations can be accounted for if we assume that the light-harvesting complex is positioned near the inner surface of the membrane in association with the tetramer. One complication with which we have not dealt is the suggestion (15) that, in addition to the absence of the light-harvesting complex, several minor components are also reduced in the mutant. Another report (35), however, suggests that this is not the case. Nevertheless, we must be careful to note that, if some minor components are also affected by the mutation, these may in part be responsible for the structural changes we have observed in the mutant. We do not feel that this is a major problem, owing to the minor nature of reductions observed in these weakly staining polypeptide bands (15).

Thus, although we are receptive to the idea that several explanations for the changes in membrane structure reported here may be developed, it seems clear that the one which presents the fewest difficulties and ambiguities is that which proposes an association between the light-harvesting chlorophyll-protein complex and the membrane-spanning tetramer.

Our results also suggest that the tetramer may represent a structural equivalent of the photosystem II reaction complex. The proposed association of the light-harvesting complex with this struc-

ture makes the identification reasonable. Other workers (28; footnote 1) have also suggested that these particles may be associated with the photosystem II reaction, primarily on the basis of the fact that these particles, like photosystem II itself, are concentrated in stacked regions of the membrane system. Studies by a number of workers (20, 30, 34) have shown that the light-harvesting chlorophyll-protein complex also follows this distribution, and fractions prepared from grana membranes are substantially enriched in the complex in comparison to fractions prepared from unstacked stromal membranes. Our results are in good agreement with these reports.

These results also suggest a manner in which the light reaction might occur across the photosynthetic membrane, summarized in Fig. 18. The light-harvesting complex should lie near the photosystem II reaction center to provide a close coupling for exciton transfer from the chlorophyll molecules of the complex to the reaction center. An excellent review by Trebst (36) has summa-

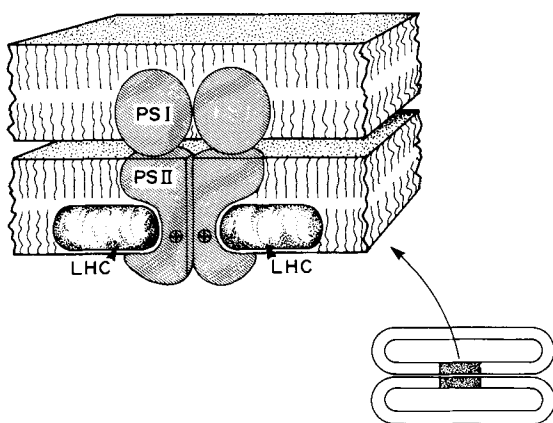


FIGURE 18 A schematic representation of how particles within the photosynthetic membrane might carry out the light reaction of photosynthesis. Small portions of two adjacent membranes in a stacked region are shown, cut open in such a way that only two subunits of the membrane spanning tetramer are seen. Evidence for the release of oxygen at the interior of the sac and the reduction of NADP^+ has been summarized by Trebst (33) and Anderson (1). The tetramer spans the membrane (19) and makes contact with small particles on the opposite membrane in stacked regions (29). Data presented in this paper have implied the location of the light-harvesting chlorophyll-protein complex of photosystem II (LHC). One possible location of the photosystem II (\oplus) reaction center is shown. Although the identity of the small particle is still uncertain, it may represent the photosystem I reaction complex (PS I).

ried evidence for the vectorial nature of electron transport. Reactions leading to the evolution of oxygen may occur at the inner surface of the membrane (18), while the final step in noncyclic electron flow, the reduction of NADP^+ , seems to occur at the surface of the thylakoid (6). If photosystem I is associated with the small particle, then the particle alignment reported by Staehelin (31) might be related to electron transfer between the photosystems.

There are uncertainties with this scheme, especially the absence of any evidence for the identity of the small particle. We anticipate, however, that work on photosystem I mutants in the near future will clarify this point. We are also unable to settle the question of whether the light-harvesting complex is exposed to the interior of the thylakoid sac, although studies with improved surface labeling techniques should be useful in this regard.

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