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Light-induced Changes in the Conformation and Configuration of the Thylakoid Membrane of *Ulva* and *Porphyra* Chloroplasts *in Vivo*

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ABSTRACT

The ultrastructural basis of light-induced transmission and light scattering changes of thalli of *Ulva* and *Porphyra* were investigated by high resolution electron microscopy and microdensitometry. The results show that upon illumination of dark thalli (*a*) a reduction in thickness of thylakoid membranes (conformational change), (*b*) a more regular ordering, and (*c*) flattening of the thylakoids (configurational changes) have occurred. An explanation for the observed conformational and configurational changes was proposed in terms of correlated changes in ionic environment and osmotic properties of chloroplasts *in vivo* which are initiated by photosynthetic reactions.

It is well established that structure and function are intimately related in chloroplasts *in vitro*, where the ionic environment and light are regulating factors in determining the size (19, 21), configuration (2, 5, 7, 17, 24), and conformation (16, 17) of isolated chloroplasts. Since study of the underlying mechanisms is facilitated by using isolated chloroplasts, such studies generally have given information upon the properties of the inner chloroplast membranes, the thylakoid membrane system, and/or the grana membrane system apart from an outer membrane. Isolated chloroplasts generally lose either part or all of their outer membrane (chloroplast envelope), and, hence, endogenous factors present in the chloroplast matrix are dissipated. There is, therefore, some difficulty in predicting *a priori* the properties of intact chloroplasts from studies *in vitro*. Indeed, Nobel (19–21) recently has studied this problem and showed that the ionic relations and efficiency of ATP synthesis are somewhat different in chloroplasts rapidly isolated from plants to preserve intact outer membranes. Likewise, it is well known from recent studies of Walker (28), Jensen and Bassham (10), and Kalberer, Buchanan and Arnon (12) that the integrity of outer membranes is necessary to preserve high rates of carbon cycle activity.

Therefore, the question how do chloroplasts behave *in vivo* with respect to the problems of ion transport and structure is important for clarifying the basic mechanisms underlying the regulation of chloroplast structure and function *in vivo*. In this regard Packer, Barnard, and Deamer (23) studied photometric properties and structural changes of chloroplasts *in vivo* under different ionic and light conditions by monitoring the transmission and light scattering changes and observing the structure by electron microscopy. This type of behavior was observed in the leaves of a number of different species of higher plants and also in the thalli of sea algae *Ulva*, *Enteromorpha*, and *Porphyra*. The latter were of particular interest because the thallus is only one or two cell layers thick. This obviates the necessity of performing vacuum infiltration in order to bring nutrients and test solutions to the leaf cells in a relatively short period of time. Moreover, the chloroplasts in the cells of these species are very large; hence the light-induced change in the photometric signal which reflects changes in chloroplast structure *in vivo* is proportionately very large with respect to the nonresponding background noise of unreacting cellular structures. Therefore, such marine algae offer considerable advantages for a thorough study of the factors which underly the control of chloroplast structure *in vivo*. The present investigation was undertaken to determine to what extent changes in chloroplast structure in *Ulva* and *Porphyra* could be predicted *in vivo* from present knowledge of the behavior of isolated chloroplasts with respect to the relation between ion transport and ultrastructure.

MATERIALS AND METHODS

Preparation of Materials. *Ulva* (either *Ulva expansa* or *Ulva lobata*) and *Porphyra* sp. were collected in the vicinity of Bodega Marine Laboratory. A section of thalli (2 × 2.5 mm) was sandwiched between two black Bakelite frames and soaked for 40 to 60 min prior to the experiments in an incubation medium in the dark at low temperature. This procedure was used to deplete or to change the ionic composition of cells and to make sufficient supply of an electron transport cofactor. Incubation media employed in this study were (*a*) sea water, (*b*) 100 mM sodium chloride, and (*c*) 100 mM sodium acetate, and they contained either 20 μM PMS[3] or 2.5 mM potassium ferricyanide as an electron transport cofactor. pH of the incubation and other conditions are indicated in the legends for each experiment.

Measurement of Transmission and 90° Light Scattering Changes. For experiments where light-induced transmission and 90° light scattering changes in the thalli were examined, the procedure of Packer, Barnard, and Deamer (23) was employed. This consists of cutting a small section of thalli and locating it between two black Bakelite frames which fit within the cylindrical cuvette of the spectrophotometer. The thallus was oriented in the cuvette

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[3] Abbreviations: PMS: phenazine methosulfate; DCMU: 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PMA: phenylmercuric acetate; ANS: 8-anilinonaphthalene-1-sulfonic acid.

at 45° to the direction of measuring and actinic beams such that interference with reflected light from the surface of the material was minimized. Transmission was measured at 180° using light from tungsten lamp filtered at 546 nm. The intensity in the dark before illumination was adjusted to read 100% on the chart paper. In some experiments the 90° light scattering change at 546 nm was also monitored simultaneously with transmission changes in a Brice-Phoenix light scattering photometer equipped with two photomultiplier tubes according to the procedure mentioned above. The red actinic light used to induce transmission and light scattering changes (as a photometric parameter of structural changes of plastids) was isolated by means of a filter between 600 and 700 nm, and its intensity was 900 ft-c. The temperature of the incubation medium was maintained at 25 C by circulating water through a jacket around the cuvette.

Electron Microscopy. In order to stop the reaction quickly and fix the structure of plastids at different states in conformation, 8% neutral glutaraldehyde (Polyscience Inc., Warrington, Pa.) was added to the incubation under the different light conditions (dark before illumination, light, and dark after illumination) to a final concentration of 1%. The thallus was then kept for at least 15 more min in the incubation medium under the same conditions; then samples were removed from cuvettes and fixation of the thalli was continued further for 1 hr in 2.5% glutaraldehyde in the presence of 50 mM phosphate buffer (pH 7.4). This was followed by 1 hr of treatment in 1% osmium tetroxide in 50 mM phosphate buffer (pH 7.4). Fixed materials were dehydrated with a graded series of alcohol, infiltrated with propylene oxide, and embedded in Epon-Araldite mixture (15) according to the schedule recommended by Johnson and Porter (11). Sections cut parallel to the surface of the thalli were stained with lead citrate and uranyl acetate (25) and observed in the electron microscope using either Simens Elmiskop II or RCA EMU-3H.

RESULTS

Photometric Studies. The initial experiments were performed to determine optimal conditions for inducing light-dependent structural changes in *Ulva* chloroplasts *in vivo*. Previous studies (23) had established that light-induced transmission, configurational, and volume changes which occur in leaves of higher plants and thalli of marine algae are enhanced by the presence of externally added electron transport cofactors. Table I shows the extent of this type of effect for transmission responses in *Ulva*. The results show that the greatest light-induced transmission decrease is obtained when the tissue is in the presence of either cyclic (PMS) or noncyclic (ferricyanide) types of electron transport cofactors. Although light-induced transmission changes occur even in the absence of electron transport cofactors, it is clear that the magnitude of the responses is considerably enhanced by the presence of such cofactors. In general ferricyanide has been found more effective in this regard when tested at optimal concentration (between 2.5 and 6 mM). In other experiments a comparison of the kinetics of the light-induced transmission changes for ferricyanide- or PMS-supported responses indicates that the rate of activation and decay of the transmission changes is also similar with both cofactors.

Table I. *Light-induced Transmission Responses of Ulva Species*

Conditions as described in "Methods and Materials" except that *Ulva* was soaked in the various media indicated in the table in the presence or absence of electron transport cofactors in the dark for approximately 1 hr before the experiments. Ferricyanide was 2.5 mM and PMS was 20 μM in the test medium. The pH of the medium was natural for sea water and pH 7 for the experiments with sodium chloride and sodium acetate media. Data are presented as the average (number of experiments).

Medium	Light-induced Transmission Decreases		
	No cofactor	PMS	Ferricyanide
	%	%	%
Sea water	1.4 (5)	2.4 (1)	11.6 (7)
NaCl (0.1 M)	2.4 (5)	5.9 (2)	11.4 (4)
NaCl + tris(0.005 M)	5.1 (5)	...	10.2 (12)
Na acetate (0.1 M)	6.5 (1)	12.3 (4)	11.9 (16)
Na acetate + tris (0.005 M)	...	8.9 (4)	7.8 (5)

It is also worth noting that maximal cofactor stimulation of the light-induced transmission responses requires soaking of the thallus for 0.5 to 2 hr at 2 C. This time is presumably required for penetration of the cofactor to the chloroplasts within the cells. In higher plant leaves the technique of vacuum infiltration is necessary to insure penetration of the cofactor to the cells (23). This procedure appears unnecessary in green algae because the *Ulva* thallus is only two cell layers thick.

These studies also show that the ability of *Ulva* to evidence light-induced chloroplast structural changes exists not only in sea water, the natural condition, but also in 100 mM solutions of various sodium salts at pH 7.

pH Dependence. A study has been made of light-induced transmission changes in the sodium acetate-ferricyanide system in the pH range 4 to 8.8. The largest light-induced responses occur in the range 6.9 to 7 2.

Inhibitors. A number of reagents known to affect light-dependent reactions associated with electron flow in chloroplasts have been examined for their effect upon the light-induced transmission changes in *Ulva*. Table II (A) shows that DCMU completely inhibits this response when a concentration in the vicinity of 13 μM is in the test medium. This concentration causes a complete reversal of the light-induced transmission change. Fifty per cent inhibition is achieved with about 5 μM DCMU.

Table II. *Action of DCMU and PMA Upon Light-induced Transmission Changes in Ulva*

Conditions as described in "Methods and Materials" except that the inhibitor was added to the test medium (100 mM sodium acetate) as follows. For DCMU, addition was made after the completion of the light-induced transmission decrease; hence the data refer to the percentage reversal of the response under conditions of continued illumination. For PMA, the substance was added in the dark approximately 1 min before illumination of the thallus with actinic light.

Concentration	Light-induced Transmission Change	
	Reversal	Inhibition
(μM)	%	%
A. DCMU		
1.67	0	
5.01	52	
6.7	83	
10.0	92	
13.4	96.5	
B. PMA		
0		0
0.3		83
3.3		58
33.3		100
33.3[1]		38

[1] PMS cofactor instead of ferricyanide.

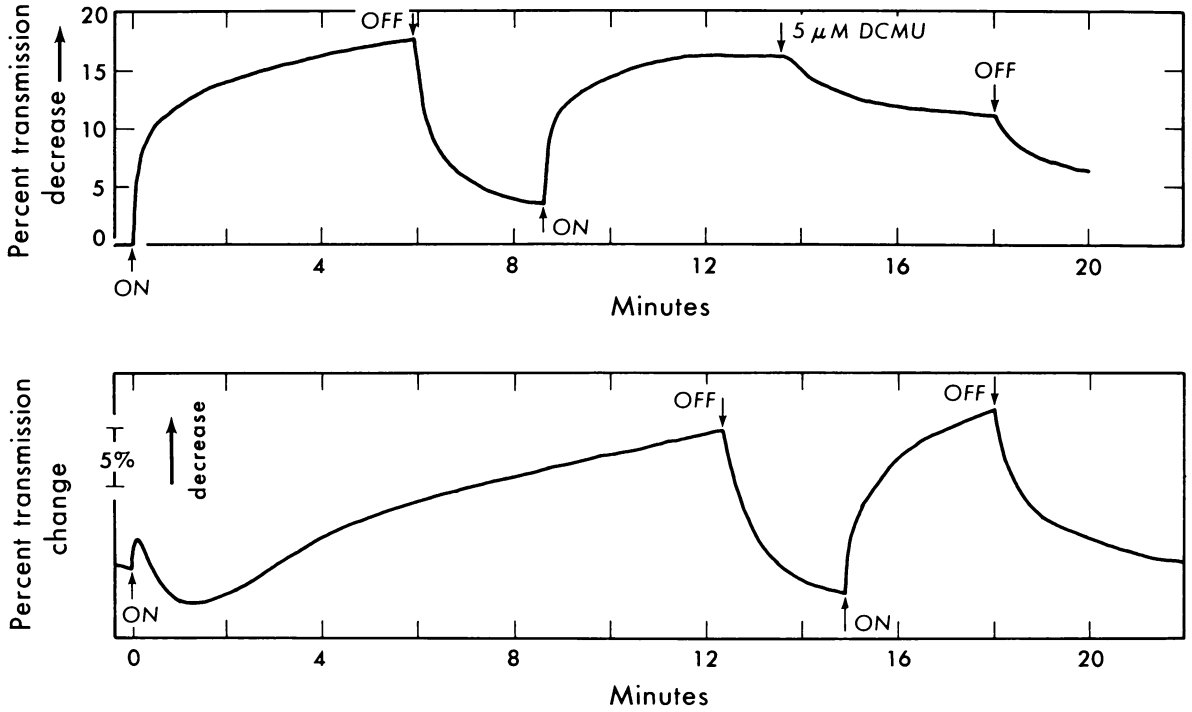

FIG. 1. Top: Usual kinetics of light-induced transmission changes in *Ulva*. Thalli were soaked in the dark at 0 C for 1 hr in the presence of 100 mM sodium acetate (pH 6.0) plus 2.5 mM ferricyanide. Other conditions as in text. Bottom: Unusual kinetics of light-induced transmission changes in *Ulva*. Thalli were soaked in the dark at 0 C for 1 hr in the presence of 4 mM tris buffer, 100 mM sodium acetate, and 20 μM PMS at pH 7.0. Other conditions as in text.

While the site of action of the organic mercurial, phenylmercuric acetate, in photosynthesis is still under investigation (Starks and Packer, unpublished), this reagent is known to inhibit both electron transport and photophosphorylation processes (27). Table II (B) shows that, in the presence of cyclic or noncyclic cofactors for electron transport, PMA, even at rather small concentrations, exerts a powerful inhibition upon the light-induced conformational change.

Kinetics of Light-induced Transmission and Light Scattering Changes. A typical example of the light-induced transmission changes in *Ulva* is shown in Figure 1 (top). Upon illumination a transmission decrease occurs which continues for several minutes. After 2 min the transmission has decreased by 15%. As the recording is continued, transmission slowly decreases somewhat further; and then, when the light is removed, a rapid increase of transmission occurs but the response is largely but not fully reversible. Hence, the slow component (observed after the first phase of the light-induced transmission decrease is completed) may reflect a secondary, perhaps osmotically induced process, involving either the chloroplast or another component of the cells. A second illumination is again accompanied by a decreased transmission of approximately the same magnitude as observed in the first illumination cycle. An addition of 5 μM DCMU under conditions of continued illumination causes a partial reversal of the transmission decrease, and, when the light is finally extinguished, a more complete reversal ensues. This type of kinetics for both rate and extent is what is usually observed when *Ulva* is examined in the presence of electron cofactors and the ionic media such as those described in Table I.

Occasionally, however, peculiar responses of the type demonstrated in Figure 1 (bottom) have been observed. Upon illumination there is first a rapid decrease in transmission which suddenly reverses itself, resulting in a temporary transmission increase. After several minutes the transmission again slowly decreases so

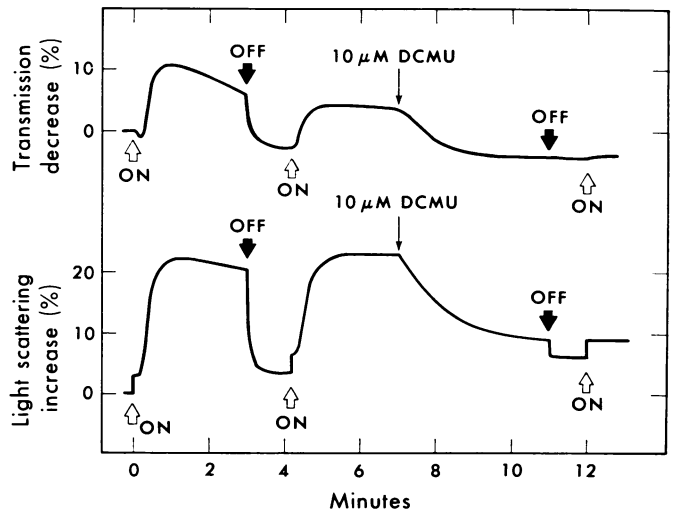

FIG. 2. Kinetics of light-induced transmission and 90° light scattering changes in *Porphyra*. Thalli were soaked in 100 mM sodium acetate (pH 6.7) containing 2.5 mM potassium ferricyanide for 1 hr in the dark, and then responses of transmission and light scattering to illumination were recorded simultaneously in the photometer.

that the over-all response of illumination is a transmission decrease. Later, when actinic light is extinguished, the typical rapid and large reversal of the transmission change occurs. A second cycle of illumination (and also with further cycles) the kinetics apparently return to the typical pattern for light-induced transmission changes as described for Figure 1 (top). Hence, this experiment shows that under some circumstances illumination induces two opposing transmission changes. One seems associated with the light reactions in the chloroplasts. The other change, the

"spontaneous" reversal observed in the light, may reflect secondary water movements and resulting osmotic changes in the cells, possibly swelling. The latter effect seems likely to be related to the incomplete reversibility of the transmission responses already noted for the "usual" kinetic responses (Fig. 1, top). This is the case with *Porphyra* thalli as mentioned below.

With *Porphyra* thalli similar kinetics of light responses to those described for *Ulva* were obtained. Illumination of the thalli in 100

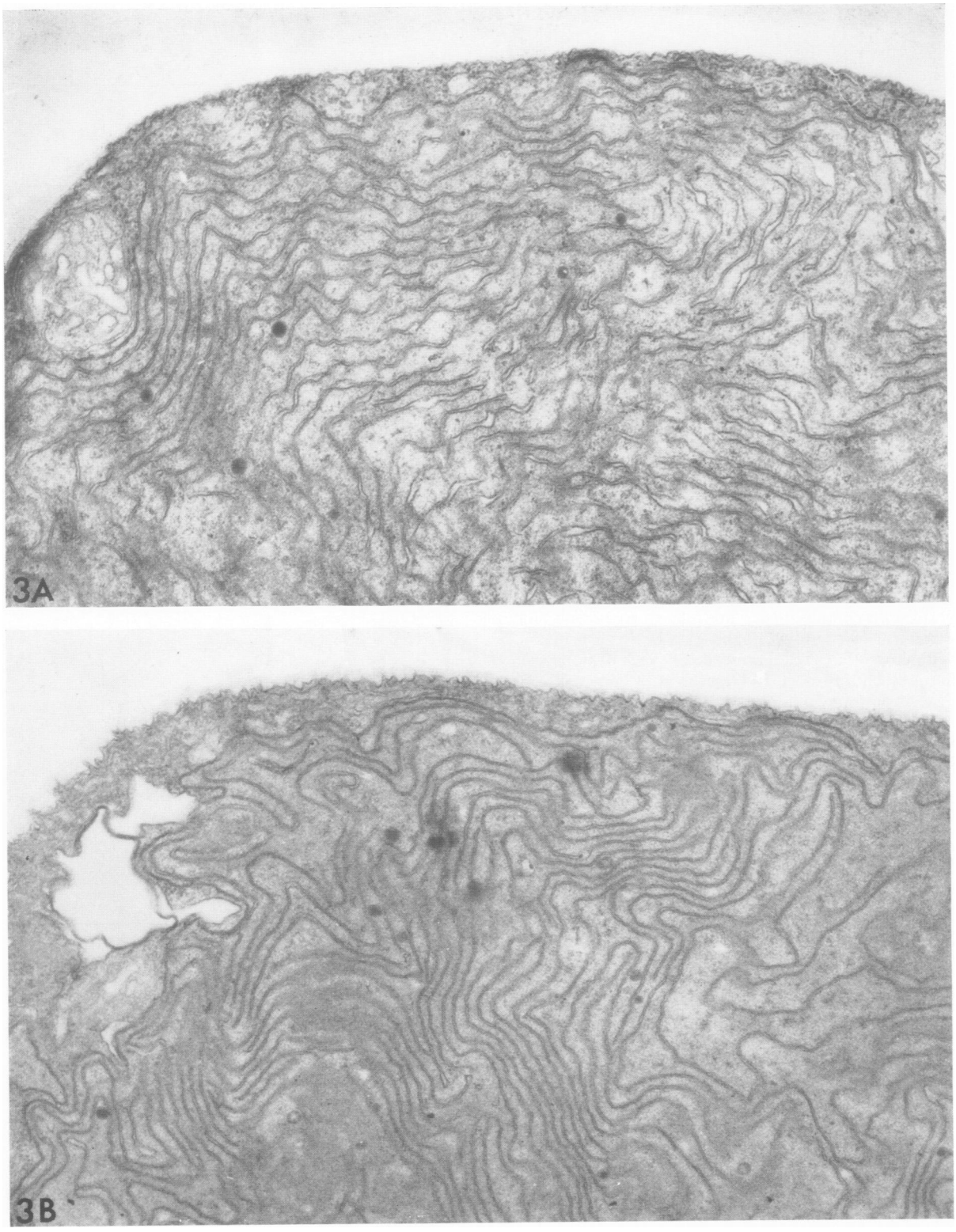

FIG. 3. Ultrastructure of *Porphyra* cell in the darkness, before illumination (A) and under continuous illumination (B). Thalli were soaked for 40 min in 100 mM sodium acetate (pH 6.6) plus 2.5 mM potassium ferricyanide in the dark and fixed successively with glutaraldehyde and osmium tetroxide as described in "Methods and Materials." For the light experiment, thallus was illuminated for 2 min by red actinic light and then fixed. × 29,200.

mM sodium acetate containing 2.5 mM potassium ferricyanide induced a rapid decrease in transmission which reached maximal level (11–15%) after 1 min (Fig. 2). The simultaneous recording of 90° light scattering showed an increase in the intensity of the light scattering upon illumination. Both changes were reversed by extinguishing the actinic light. In some cases, as shown in Figure 2, light-induced transmission decrease was preceded by a small negative response (increase), which corresponded to short lag phase in light scattering increase. A second cycle of transmission and light scattering changes was induced by repeated illumination

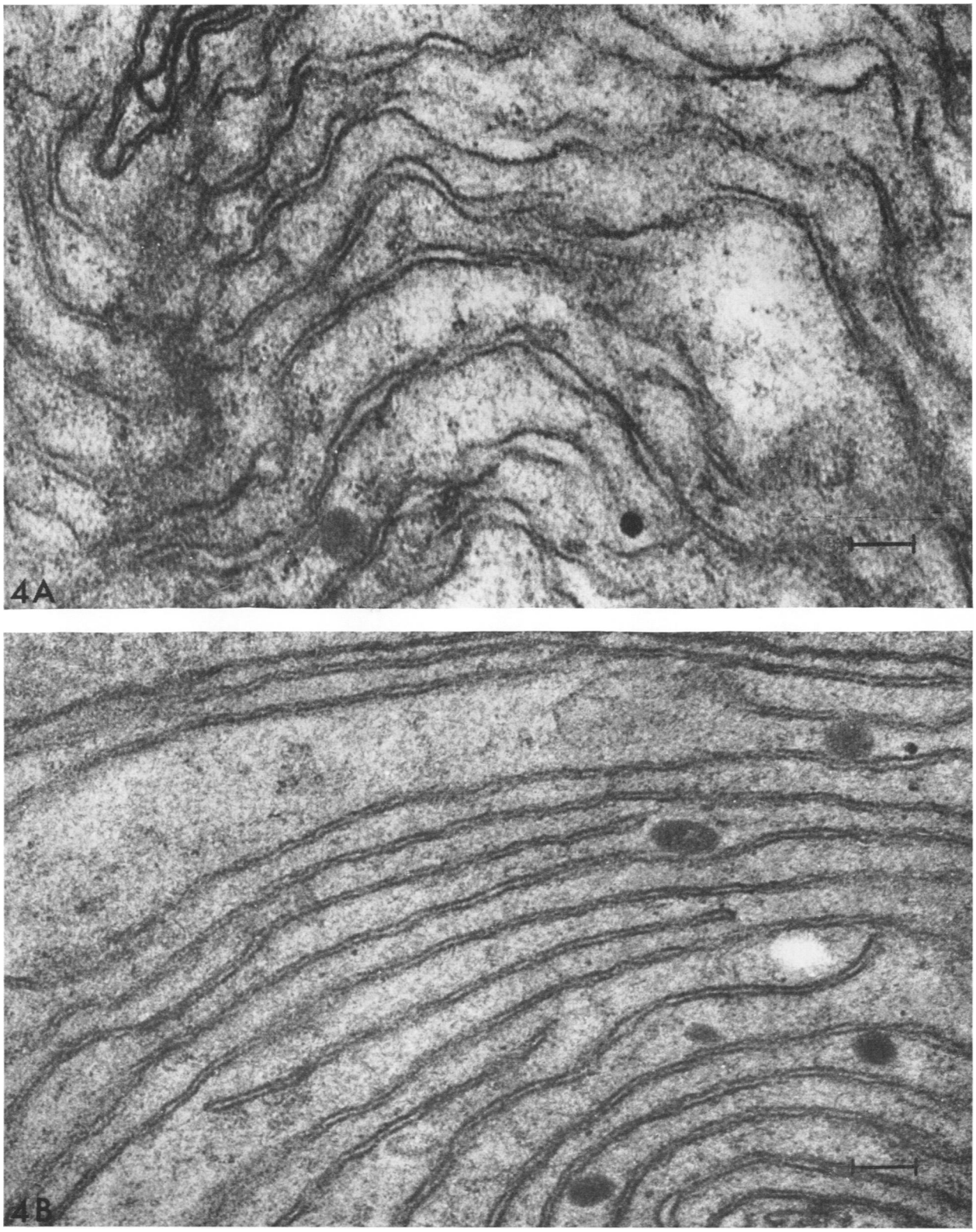

FIG. 4. Ultrastructure of the thylakoids in *Porphyra* chloroplast in the dark (A) and light (B). Conditions for incubation and illumination as described in Fig. 3. × 109,000.

with normal fashion. DCMU, 10 μM, added to the incubation medium under condition of continued illumination reversed almost completely both transmission and light scattering levels, and in the presence of DCMU no further responses of these two photometric parameters to illumination were observed.

Ultrastructural Studies of Thylakoid Membrane. It is reasonable to assume that many changes in cell structure which give potent effects on photometric properties of organelles might be induced upon illumination. Among these are (*a*) location in the cell, (*b*) configuration (or shape), (*c*) volume (or size) and (*d*) internal

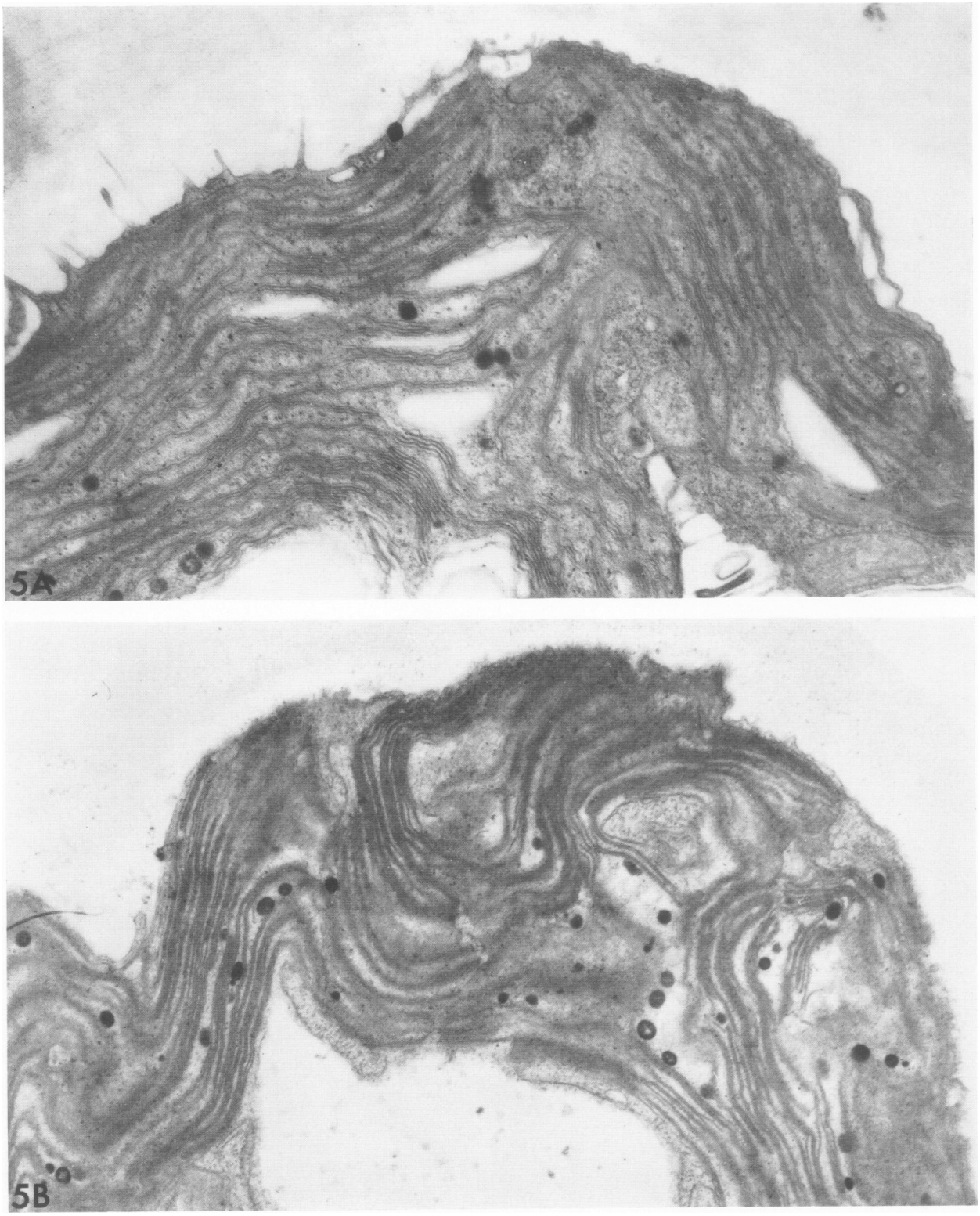

FIG. 5. Ultrastructure of *Ulva* cell in the darkness, before illumination (A) and under continuous illumination (B). Thalli were preincubated for 40 min in 100 mM sodium acetate (pH 6.6) plus 20 μM PMS in the dark and fixed with glutaraldehyde and osmium tetroxide as described in "Methods and Materials." For the light experiment, thallus was illuminated for 5 min by red light and then fixed. × 26,700.

membrane structures (conformation) of plastids or mitochondria, or both. These are the questions which can be answered by examining the structural states of these organelles by electron microscopy and correlating the results with the corresponding metabolic states that are regulated by light conditions. Indeed, it has been established that the membrane system of chloroplasts *in vitro* (5, 7, 17) and *in vivo* (13, 21, 23) becomes flattened (photoshrinkage) upon illumination, which is accompanied by changes in internal structure of thylakoid membranes (16, 17). Hence, a study of the microstructure of thylakoid membranes in the plastids of *Ulva* and *Porphyra* was undertaken during different states of light responses.

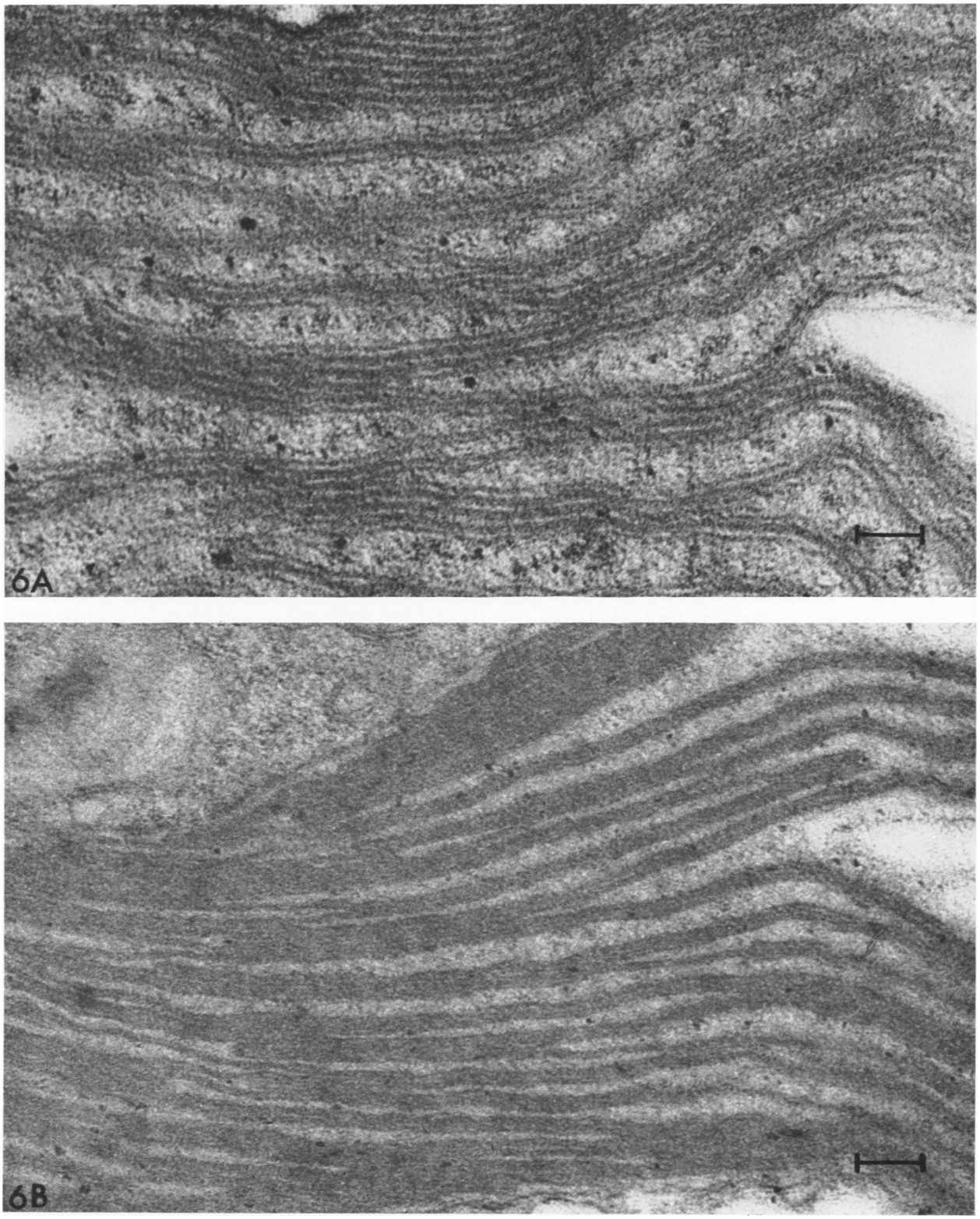

FIG. 6. Effect of illumination on ultrastructure of thylakoids in *Ulva* chloroplast. A: Dark incubation, before illumination; B: 4 min illumination; C: 6 min in the darkness after 4 min illumination. × 109,000.

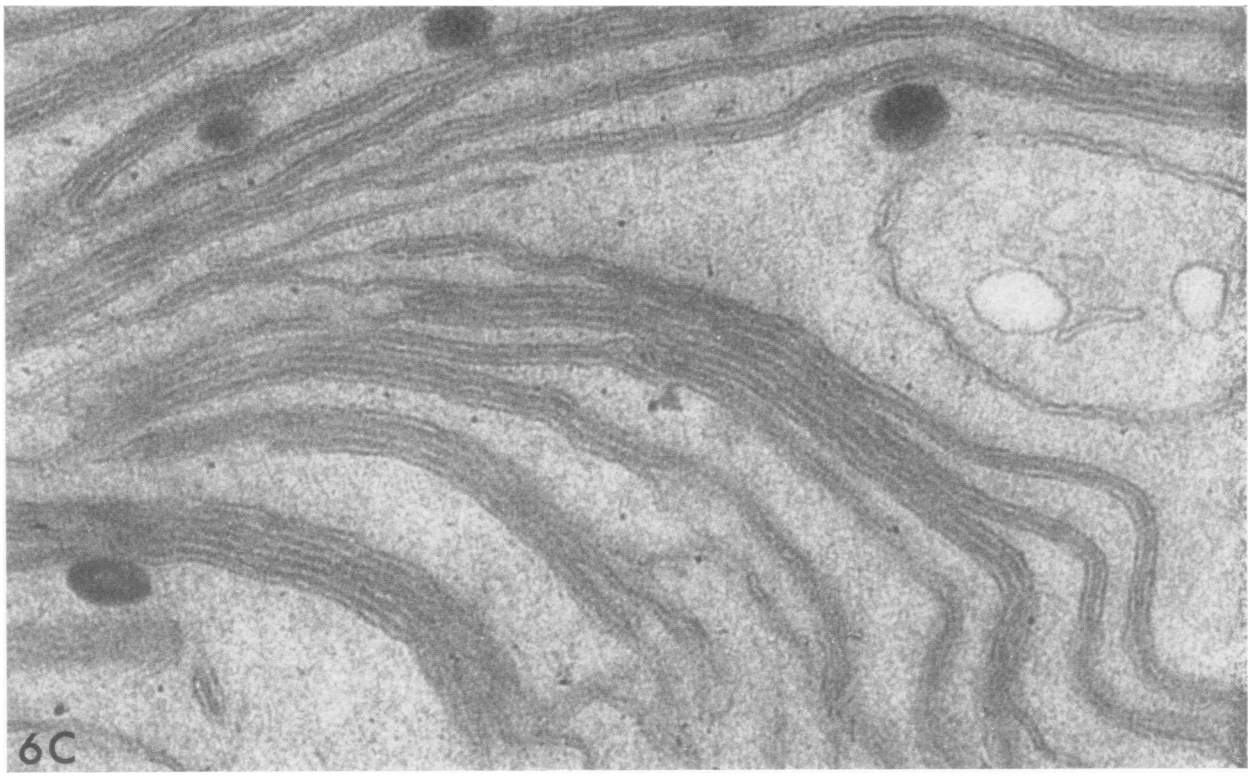

Figure 3A shows a part of cell of *Porphyra* thalli which was incubated in 100 mM sodium acetate, pH 6.6, containing 2.5 mM potassium ferricyanide in the dark. The cell is occupied by a single large plastid, and mitochondria appearing in a swollen configuration are also observed in cytoplasm at the periphery of the cell. The membrane system in the plastid consists of parallel running, undulating thyalkoids which seem to be in a swollen state. The intraspace of the thylakoids is so wide that the two walls (thalakoid membranes) of a single thylakoid are easily recognized even at such low magnification. Striking changes in the configuration of thylakoid occur when the thallus is illuminated by red light (Fig. 3B) Thylakoids turned to a more stretched and flattened form, and, hence, it became more difficult to resolve the two walls of a thylakoid seen in this figure at low magnification. Mitochondria under the condition of continued illumination seem to be in a more contracted form. The structure of the thylakoids of *Porphyra* plastids in different light conditions was compared at higher magnificaton in Figures 4A and 4B in which the light-induced configurational change of the thylakoids from a swollen, wavy form to a stretched or contracted state is more clearly shown. Distance between two thylakoid membranes of a single thylakoid (measured as center-to-center distance of the membrane) varies widely from 125 to 700 A for the case in the dark (Fig. 4A), whereas the values for illuminated plastids comes into a narrow variation between 95 and 125 A (Fig. 4B). By comparing these two figures with close inspection of the dimension of the thylakoid membrane, reduction in thickness of the membrane can be recognized after illumination. It is also noted that distribution of stroma substance is more homogeneous in the plastid in the light.

Light-induced flattening or contraction of thylakoid membrane system was demonstrated also for the chloroplasts of green algae, *Ulva* (Figs. 5 and 6). In *Ulva* chloroplasts two or more large thylakoids are organized into grana-like construction (stack of the thylakoids), though it is larger and more ill defined than the grana in the chloroplast of higher plants. This is clearly observed in the chloroplasts of thallus incubated in the dark (Fig. 5A), since under such condition the thylakoid is in a swollen configuration. When the thallus is illuminated, there occur dramatic changes in chloroplast structure (Fig. 5B); the thylakoids stretched, and thylakoid stacks became very tightly packed. This observation also suggests shrinkage of chloroplast as a whole, but it cannot be demonstrated so evidently by electron microscopy because of irregular shape of a large chloroplast in the *Ulva* cell. In the chloroplasts under dark incubation two thylakoid membranes are recognized in "partitions" in the membrane stacks, and the intraspace of the thylakoid can be seen as a clear zone between the membranes (Fig. 6A). When the chloroplasts are illuminated, the stacking of the thylakoids becomes so compact that intraspace of the thylakoids can hardly be resolved even on the photographs at higher magnification (Fig. 6B). The thickness of the thylakoid membranes was also reduced. When the thallus was again transferred into the dark, flattening of the thylakoid which was induced upon illumination was reversed completely, resulting in a similar configuration of thylakoids to that seen in the dark before illumination (Fig. 6C). Now the intraspace of the thylakoid becomes wider and the individual thylakoid membranes can be clearly observed.

Since a light-induced change in the thickness of thylakoid membrane of both *Porphyra* and *Ulva* plastids was noted on electron micrographs, this was further analyzed by means of microdensitometry of electron microscope negatives to obtain more precise and quantitative information. The areas of thylakoids showing the sharpest profiles were selected and scanned perpendicular to the direction of the membrane by a microbeam of 50 μ diameter on the microdensitometer. The results summerized in Fig. 7 show very clearly the light-induced thickness decrease of thylakoid membrane and also spacing decrease. The values (with standard deviation) are the average of more than 30 densitometry traces, which were obtained by scanning several selected areas of thylakoids in different electron microscope negatives taken at the same magnification ($\times$ 20,000). In *Porphyra* plastids in the dark before illumination the thickness (half-width) of the thylakoid membrane was 64 $\pm$ 8 A, and this value was reduced to 49 $\pm$ 4 A upon illumination, resulting in a 23% decrease. In the case of *Ulva*

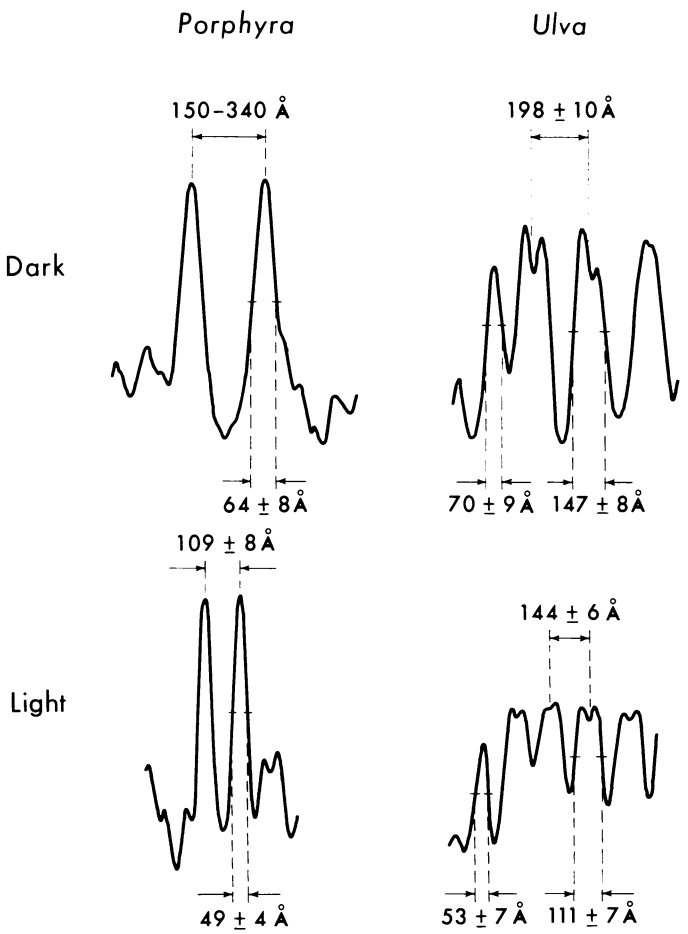

FIG. 7. Comparison of the thickness and spacing of the thylakoid membrane in *Porphyra* and *Ulva* chloroplasts *in vivo* under different conditions of illumination. Thalli were incubated as described in Figures 3 and 5. Photodensitometric curves were obtained by traversing the thylakoid membranes on electron microscope negatives by microbeam using a double beam Recording Microdensitometer MK IIIC (Joyce, Loeble and Co., Ltd.). The values (± standard deviation) of spacing (center-to-center distance) and thickness (half-width) of the thylakoid membranes are the average of more than 30 traces.

chloroplasts, a reduction of the membrane thickness estimated at partition (two apposed thylakoid membranes) was 20%, and spacing (center-to-center distance) of stacked thylakoid membranes decreased from 198 ± 10 A in the dark to 144 ± 6 A in the light. The membrane thickness measured with single thylakoid membrane was 70 ± 9 A in the dark and 53 ± 7 A for illuminated chloroplasts.

DISCUSSION

It has clearly been established in the present investigation that electron transport-dependent changes in the structure of thylakoid membranes occur in chloroplasts *in vivo* of *Ulva* and *Porphyra* thalli. The most obvious changes observed by electron microscopy are in those in configuration of the thylakoid membrane system, *i.e.*, a decrease in intraspace or flattening of thylakoid upon illumination, which is reversed in the dark. Photometric evidence observed is, therefore, reasonably interpreted as derived from such kinds of changes in chloroplast structure. Moreover, it has been shown in isolated chloroplasts that transmission changes correlate most closely with the changes in spacing of the thylakoid membrane (17). A second type of configurational change is that of shape and ordering of thylakoids. This was shown more evidently in the case of *Porphyra* in which thylakoid changed from an irregular or undulating shape to a more stretched and ordered array with more regular intervals between membranes following illumination. It would appear that these configurational changes lead to the changes in volume, size, and shape of the chloroplasts observed *in vivo*. Thus, these results from marine algal chloroplasts confirm and extend previous studies of Kushida, Itoh, Izawa, and Shibata (13) and Packer, Barnard, and Deamer (23) showing light-induced flattening of chloroplasts *in vivo*.

In both *Ulva* and *Porphyra*, when flattening of the thylakoid has occurred, a more fundamental change at the level of molecular structure or conformation is also observed in that a decrease in the thickness of the thalakoid membrane itself is observed. A thickness change of the thylakoid membrane was first observed by Murakami and Packer (16) in isolated chloroplasts when the chloroplast shrinkage was induced upon illumination. Subsequently Murakami and Packer (17) studied extensively this phenomenon using various means, including artificial pH change of the medium and ANS fluorescence probe, to induce and monitor the conformational changes of thylakoid membrane. All of the conditions which induced spacing change caused membrane thickness change with one notable exception, osmotic treatment. When the chloroplasts were titrated with HCl, binding of H^+ to the thylakoid membrane occurred in the pH range between 7 and 4.5, and 23% decrease of membrane thickness was observed. From these results a protonation model was presented to interpret membrane conformational changes observed. Protonation of negatively charged groups of molecules in the membrane (*e.g.*, β- and γ-carboxyl, secondary phosphate and imidazole groups) causes changes in conformation of the molecules and molecular interactions, and hydration of the membrane, resulting in the decrease of the membrane thickness. This provided experimental basis for a protonation concept of the membrane which was postulated by Dilley and Rothstein (8), and Deamer, Crofts, and Packer (5) as a cause of conformatonal change of thylakoid membrane. The present study *in vivo* showing similar changes to those in chloroplasts *in vitro* may have a similar explanation. This view is further supported by the fact that conformational changes in thylakoid membranes were demonstrated in spinach chloroplasts *in vivo* (unpublished observation, Murakami and Packer).

The experiments performed in sodium acetate medium containing electron transport cofactors lead to conformational changes in thylakoid membrane and seem to simulate natural conditions since photometric responses are also observed in ordinary sea water, especially if supplemented with an electron transport cofactor.

The question, therefore, is where, when, and how does this hierarchy of structural changes arise in chloroplasts *in vivo*? The scheme shown in Figure 8 is an attempt to provide a plausible explanation for these questions. It is clear that a partial answer to these questions is the requirement for light and electron transport cofactors. Therefore, the next question is what are the steps intermediate between electron transport and a possible change in structure of the molecules in the thylakoid membrane? It would appear that the answer to this question involves an understanding of the electron transport-dependent ion movement initiated by light in chloroplast *in vivo*. Studies with isolated chloroplasts have demonstrated a light-dependent hydrogen ion uptake (5, 18, 22) and also movements of other ions in chloroplasts (2, 6, 9, 20, 22). For example, illuminated chloroplasts show an effiux of weak acid anion-type substances which move out of the chloroplast (2, 6). In this case, light-induced hydrogen ion uptake increases acidity inside, causing an increase in the concentration of undissociated acids inside; and the increased undissociated acids are freely diffusible and move out of the chloroplasts, carrying with them osmotically active material, causing osmotic collapse of thylakoid membranes. This type of mechanism should occur *in*

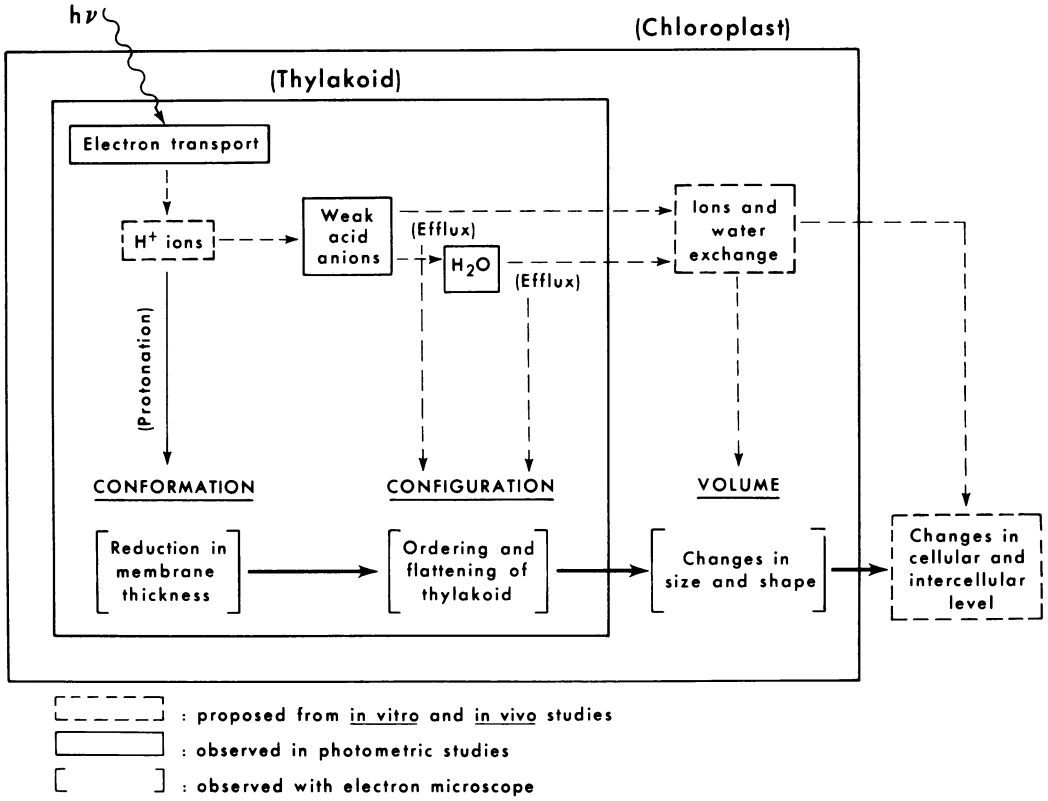

FIG. 8. Hypotheses for structural changes in thylakoid membrane *in vivo*.

vivo because weak acid anion substances are the common type of carbon compounds that would be expected to be present in appreciable concentration within chloroplasts. Moreover, there is evidence that the light-induced hydrogen ion uptake manifested by chloroplasts *in vitro* is also present in chloroplast *in vivo*. West and Wiskich (30) have demonstrated that pea chloroplasts isolated with intact outer membranes retain light-induced hydrogen ion uptake activity. Cummins, Strand, and Vaughan (3, 4) and Ben-Amotz and Ginzburg (1) have recently found a light-induced hydrogen ion uptake activity in thalli of *Ulva* species and intact cells of halophilic alga *Dunaliella*, respectively. These studies taken together with the present results strongly suggest that this sort of activity probably also occurs in the chloroplasts of *Ulva* and *Porphyra* species under the conditions employed in this study. If such activity is present, localized changes in pH could change the molecular structure of the thylakoid membrane according to mechanisms that have recently been shown to occur for isolated chloroplasts (17) and mitochondria (31), where pH-dependent molecular conformational changes in membrane structure have been observed by various indirect photometric techniques and correlated electron microscopy Therefore, it seems reasonable to propose the relationship between light and the gross morphological changes in *Ulva* and *Porphyra* chloroplasts *in vivo* in the sequence as indicated by the arrows in Figure 8.

There have been a number of studies in which light-induced ion movement in intact algal cells has been investigated. Scott and Hayward (26) and West and Pitman (29) have obtained evidence for the close correlation between cellular metabolism, especially photosynthetic reactions, and sodium and potassium effiux in *Ulva lactuca*. In these instances, the ion movements observed were very slow, since the experiments were not carried out to determine unidirectional translocation. Recently, Cummins, Strand, and Vaughan (3, 4) introduced an elegant technique with which the transport of ions from one external compartment to another one through the algal thallus can be followed, and they have demonstrated rapid light-induced extrusion of sodium and other cations like Rb^+ and Sr^{2+} from the thallus of *Ulva expansa* which is accompanied by hydrogen ion uptake. From these results it is very reasonable to assume that there is an ion exchange across chloroplast envelope, tonoplast, and cell membrane in intact *Ulva* and *Porphyra* cells, resulting in the changes in ionic environment and osmotic properties which lead to volume, size, and shape changes of the chloroplasts *in vivo* and whole cells.

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LITERATURE CITED

1. BEN-AMOTZ, A. AND B. Z. GINZBURG. 1969. Light-induced proton uptake in whole cells of *Dunaliella parva*. Biochim. Biophys. Acta 183: 144–154.
2. CROFTS, A. R., D. W. DEAMER, AND L. PACKER. 1967. Mechanisms of light-induced structural changes in chloroplasts. II. The role of ion movements in volume changes. Biochim. Biophys. Acta 131: 97–118.
3. CUMMINS, J. T., J. A. STRAND, AND B. E. VAUGHAN. 1966. Sodium transport in *Ulva*. Biochim. Biophys. Acta 126: 330–337.
4. CUMMINS, J. T., J. A. STRAND, AND B. E. VAUGHAN. 1969. The movement of H^+ and other ions at the onset of photosynthesis in *Ulva*. Biochim. Biophys. Acta 173: 198–205.
5. DEAMER, D. W., A. R. CROFTS, AND L. PACKER. 1967. Mechanisms of light-induced structural changes in chloroplasts. I. Light-scattering increments and ultrastructural changes mediated by proton transport. Biochim. Biophys. Acta 131: 81–96.
6. DEAMER, D. W. AND L. PACKER. 1969. Light-dependent anion transport in isolated spinach chloroplasts. Biochim. Biophys. Acta 172: 539–545.
7. DILLEY, R. A., R. B. PARK, AND D. BRANTON. 1967. Ultrastructural studies of light-induced chloroplast shrinkage. Photochem. Photobiol. 6: 407–412.
8. DILLEY, R. A. AND A. ROTHSTEIN. 1967. Chloroplast membrane characteristics. Biochim. Biophys. Acta 135: 427–443.
9. DILLEY, R. A. AND L. P. VERNON. 1965. Ion and water transport processes related to the light-dependent shrinkage of spinach chloroplasts. Arch. Biochem. Biophys. 111: 365–375.
10. JENSEN, R. G. AND J. A. BASSHAM. 1966. Photosynthesis by isolated chloroplasts. Proc. Nat. Acad. Sci. U.S.A. 56: 1095–1101.

11. JOHNSON, U. G. AND K. R. PORTER. 1968. Fine structure of cell division in *Chlamydomonas reinhardi*, basal bodies and microtubules. J. Cell Biol. 38: 403–425.
12. KALBERER, P. P., B. B. BUCHANAN, AND D. I. ARNON. 1967. Rates of photosynthesis by isolated chloroplasts. Proc. Nat. Acad. Sci. U.S.A. 57: 1542–1549.
13. KUSHIDA, H., M. ITOH, S. IZAWA, AND K. SHIBATA. 1964. Deformation of chloroplasts on illumination in intact spinach leaves. Biochim. Biophys. Acta 79: 201–203.
14. MACROBBIE, E. A. C. 1965. The nature of the coupling between light energy and active ion transport in *Nitella translucens*. Biochim. Biophys. Acta 94: 64–73.
15. MOLLENHAUER, H. H. 1964. Plastic embedding mixture for use in electron microscopy. Stain Technol. 39: 111–114.
16. MURAKAMI, S. AND L. PACKER. 1969. Reversible changes in the conformation of thylakoid membranes accompanying chloroplast contraction or expansion. Biochim. Biophys. Acta 180: 420–423.
17. MURAKAMI, S. AND L. PACKER. 1970. Protonation and chloroplast membrane structure. J. Cell Biol. In press.
18. NEUMANN, J. AND A. T. JAGENDORF. 1964. Light-induced pH changes related to phosphorylation by chloroplasts. Arch. Biochem. Biophys. 107: 109–119.
19. NOBEL, P. S. 1967. Chloroplast shrinkage and increased photophosphorylation *in vitro* upon illuminating intact plants of *Pisum sativum*. Biochim. Biophys. Acta 153: 170–182.
20. NOBEL, P. S. 1969. Light-induced changes in the ionic content of chloroplasts in *Pisum sativum*. Biochim. Biophys. Acta 172: 134–143.
21. NOBEL, P. S., D. T. CHANG, C. WANG, S. S. SMITH, AND D. E. BARCUS. 1969. Initial ATP formation, NADP reduction, CO_2 fixation, and chloroplast flattening upon illuminating pea leaves. Plant Physiol. 44: 655–661.
22. PACKER, L., J. M. ALLEN, AND M. STARKS. 1968. Light-induced ion transport in glutaraldehyde-fixed chloroplasts: Studies with nigericin. Arch. Biochem. Biophys. 128: 145–152.
23. PACKER, L., A. C. BARNARD, AND D. W. DEAMER. 1967. Ultrastructural and photometric evidence for light-induced changes in chloroplast structure *in vivo*. Plant Physiol. 42: 283–293.
24. PACKER, L., D. W. DEAMER, AND A. R. CROFTS. 1966. Conformational changes in chloroplasts. Brookhaven Symp. Biol. 19: 281–302.
25. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17: 208–213.
26. SCOTT, G. T. AND H. R. HAYWARD. 1954. Evidence for the presence of separate mechanisms regulating potassium and sodium distribution in *Ulva lactuca* J. Gen. Physiol. 37: 601–620.
27. SIEGENTHALER, P.-A. 1966. Phenylmercuric acetate and phosphate control of light-dependent shrinkage and reactions in chloroplasts. Physiol. Plant. 19: 437–447.
28. WALKER, D. A. 1965. Correlation between photosynthetic activity and membrane integrity in isolated pea chloroplasts. Plant Physiol. 40: 1157–1161.
29. WEST, K. R. AND M. G. PITMAN. 1967. Ionic relations and ultrastructure in *Ulva lactuca*. Austr. J. Biol. Sci. 20: 901–914.
30. WEST, K. R. AND J. T. WISKICH. 1968. Photosynthetic control by isolated pea chloroplasts. Biochem. J. 109: 527–532.
31. WRIGGLESWORTH, J. M. AND L. PACKER. 1970. pH dependent changes in mitochondrial membrane structure. J. Bioenergetics. In press.