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Light-dependent absorption and selective scattering changes at 518 nm in chloroplast thylakoid membranes

(light scattering/518 nm absorbance change/conformational change)

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ABSTRACT **The light-induced absorbance change at 518 nm of isolated chloroplasts consists of a rapid phase, and a slow phase which is complete in about 20 sec. The slow component of the 518 nm absorbance change correlates with the light-induced change in 90° light scattering at 518 nm. Both show a similar time course, similar pH dependence with a maximum at pH 6.0, and similar sensitivity to inhibitors and to treatment of the chloroplasts with a low concentration of glutaraldehyde. Their light *minus* dark difference spectra are similar with maxima at about 520 nm. It is concluded that they are manifestations of the same phenomenon, and the slow absorbance increase at 518 nm is due to enhanced scattering. It is proposed that the light-induced changes in scattering at 518 nm reflect alterations in selective dispersion, due to proton uptake and conformational changes in the chloroplast thylakoid membrane.**

Isolated chloroplasts exhibit reversible light-dependent structural changes, which can be monitored by electron microscopy and by changes in packed volume, transmittance, and 90° light scattering (1–8). Similar changes have also been observed with chloroplasts *in vivo* (5, 9–11). Electron microscopy has demonstrated a thinning of the chloroplast thylakoid membrane on illumination (4). The light-induced volume decrease (shrinkage) of chloroplasts and the associated increases in absorbance and 90° light scattering are dependent on photosynthetic electron flow. They are inhibited by uncouplers of photophosphorylation (5, 12). The increase in light scattering parallels the proton uptake which is observed on illumination of chloroplasts (13). It was generally supposed that the increase in light scattering reflected the decrease in chloroplast volume, but further studies (14) showed that this was not the case when chloride was the only anion present in the medium. It was suggested that scattering was influenced by protonation and an increased refractive index of the chloroplast thylakoid system (14), or by changes in the optical properties of the stacked thylakoids (7).

In the work described in this paper, we examined light-induced 90° scattering by isolated chloroplasts in relation to the light-induced absorbance change at 518 nm. It appears that the slow kinetic component of the absorbance increase is caused by an increase in selective scattering which is dependent on the refractive index difference between the chromophore-containing particles of the chloroplast and their environment.

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid.

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MATERIALS AND METHODS

Intact chloroplasts were isolated from hydroponically grown spinach plants, essentially by the method of Reeves and Hall (15). Detached leaves (20 g) were preilluminated for 15 min at 4°C in white light (16) and ground in a Sorvall Omnimixer for 4 sec at 90% of line voltage (230 V) in a medium containing 0.33 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 2 mM isoascorbate, 0.4% bovine serum albumin, and 50 mM 2-(*N*-morpholino)ethanesulfonic acid (Mes) buffer, pH 6.5. The brei was filtered through four layers of Miracloth and the chloroplasts were sedimented by centrifugation for 40 sec at 2000 × *g*. The chloroplasts were washed once in isolation medium and resuspended in a medium containing 0.33 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 0.4% bovine serum albumin, and 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) buffer, pH 7.5, to give a chlorophyll concentration of 1.5–2.5 mg/ml.

Light-induced absorbance changes were measured with an Aminco-Chance dual-wavelength spectrophotometer (American Instrument Co., Maryland), equipped with a side-illumination attachment. The assay medium contained 0.1 M sorbitol, 4 mM $MgCl_2$, 20 mM NaCl, 10 mM K_2HPO_4, 2 mM EDTA, 50 mM Hepes, adjusted to the desired pH, and chloroplasts equivalent to 50 μg/ml of chlorophyll. Below pH 6.5, 50 mM Mes or 50 mM succinate was used as buffer. Chloroplasts suspended in 0.1 M sorbitol are no longer intact, but they show good photosynthetic control ratios (17). Actinic light ($\lambda > 630$ nm) was provided by a 650 W tungsten iodine lamp, and filtered through a 3 cm layer of water and Corning 2-58 and Wratten 29 filters. The intensity was 20 mW cm^{-2}. A Corning 4-96 filter prevented actinic light from reaching the photomultiplier. The temperature of the cuvette (1 cm pathlength) was controlled to 20°C. The amplitudes of the light-induced absorbance change were reproducible to ±5% both for the spike and the steady-state.

90° light scattering was measured in a corrected fluorescence spectrometer (18) using standard 1 cm cuvettes but with all four sides of clear glass. The two monochromators of the spectrofluorimeter were fixed at identical wavelengths. Appropriate corrections were made for scattering by the buffer.

Light-induced changes in 90° scattering were measured with high intensity red light as the actinic source (Corning 2-64 filter, $\lambda > 660$ nm, intensity 20 mW cm^{-2}) propagated in the direction opposite to that of the measuring beam. The photomultiplier was protected by a Corning 4-72 blue-green filter, and no breakthrough of actinic light was detectable. Scattering measurements were corrected for the absorption of the filter. The concentration of chloroplasts (chlorophyll, 1.5 μg/ml) was kept low to approximate to single scattering

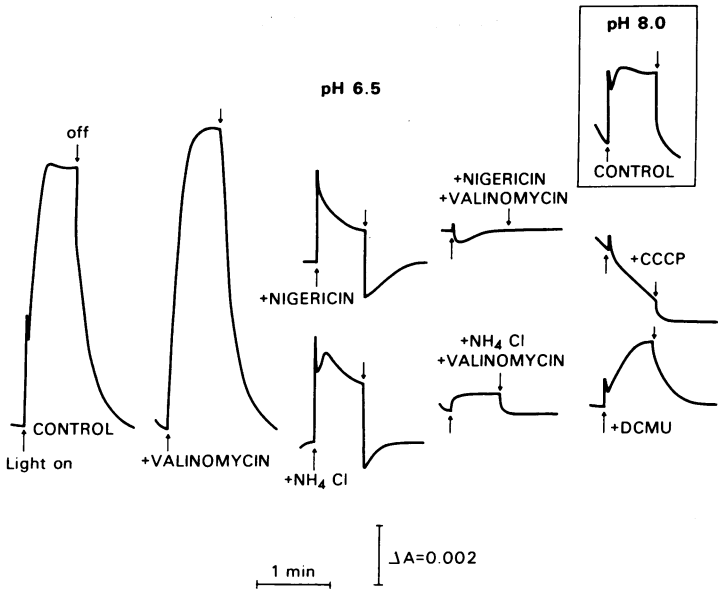

FIG. 1. Light-induced absorbance changes at 518 nm of spinach chloroplasts. Reference wavelength, 540 nm. Assay medium as given under *Materials and Methods*. Chlorophyll concentration, 50 μg/ml. Red actinic light (Corning 2-58 and Wratten 29 filters) of saturating intensity, 20 mW cm^{-2}. Upward arrow, light on; downward arrow, light off; pH of assay medium, 6.5 or 8.0 (insert). Concentrations of ionophores, uncouplers and inhibitors: valinomycin, 0.3 μM; nigericin, 0.3 μM; NH_4Cl, 2 mM; CCCP, 3 μM; DCMU, 2 μM.

conditions. The assay media were the same as used for measurements of absorbance changes. Scattering changes were reproducible to ±5%.

Scattering measurements at angles of 45° and 135° were made in a cylindrical cell, 1.5 cm in diameter. The direction of the incident beam is considered as 0°. The cell holder was fitted with a 1 mm entrance slit and 1 mm exit slits at 45° and 135° to eliminate stray light. Front surface mirrors directed the scattered light through equal pathlengths onto the entrance slit of the measuring monochromator. The pathlengths at 45° and 135° were adjusted to give equal fluorescence intensities from a dilute solution of fluorescein in ethanol with excitation at 470 nm and detection at 518 nm.

The dissymmetry ratio (I_{45}/I_{135}), which is defined as the ratio of the intensity of light scattered at 45° (forward scatter) to the intensity scattered at 135° (back scatter) was corrected for the scattering of a cell containing buffer. These scattering measurements were made in the same media as used for the 518 nm absorbance changes, but at chloroplast concentrations equivalent to 0.75–6.0 μg/ml of chlorophyll.

Proton uptake by glutaraldehyde-treated chloroplasts was measured with a glass electrode in an assay medium containing 2 mM Hepes, pH 6.5, instead of 50 mM Hepes.

RESULTS

Light-induced absorbance change at 518 nm

The form and time-course of the light-induced absorbance change of chloroplasts at 518 nm in saturating actinic light are shown in Fig. 1. At pH 8.0, the signal consisted of an initial rapid rise, followed by a partial decline and then a slow rise to a steady-state level which was complete in 15–30 sec. On lowering the pH to 6.5, the magnitude of the fast component (spike) was increased by about 50%, but there was a dramatic increase in the amplitude at the steady-state. The effects of various photosynthesis inhibitors at pH 6.5 are shown. Similar effects were observed at pH 8.0, although the extents of the absorbance increases were smaller than at the lower pH. Valinomycin, an ionophore which increases the permeability of membranes to K^+, abolished the spike but appeared to have little effect on the steady-state level. In contrast, nigericin, which exchanges K^+ for H^+ across membranes, did not inhibit the formation of the spike, but abolished the slow rise to the steady-state. Ammonium chloride had little effect on the spike, but substantially inhibited the slow rise. Valinomycin in combination either with ammonium chloride or nigericin strongly inhibited both fast and slow components. Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) also had a strong inhibitory effect on both components. The effects of these treatments on the spike of the 518 nm response are in agreement with earlier studies (19, 20) and support the conclusion that the fast component is an indicator of an electric field in or across the chloroplast thylakoid membrane (19, 21). It is apparent from the present work that the steady-state level is not inhibited by valinomycin and, therefore, it does not correlate with a membrane potential across the thylakoid membrane. On the other hand, it is strongly inhibited by uncouplers of photophosphorylation which destroy the proton gradient across the thylakoid membrane. In the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), an inhibitor of electron transport near photosystem 2, both the spike and steady-state level of the 518 nm response were inhibited about 75%.

Light *minus* dark difference spectra of the spike and steady-state level are shown in Fig. 2. Both components show similar spectra with maxima at about 518 nm.

90° light scattering

Chloroplasts at pH 6.5. show a reversible light-induced increase in 90° scattering at 518 nm with a time-course which resembles that of the slow component of the 518 nm absorbance change (Fig. 3). Light-induced 90° scattering is completely abolished by 0.3 μM nigericin, but valinomycin has no effect. DCMU (0.6 μM) inhibited the scattering by 80%. The effect of pH on the light-induced 90° scattering is shown in Fig. 4A. The result is in agreement with that of Deamer *et al.* (14). Maximum response occurs at pH 6.0 and there is a steep decline on either side of the maximum.

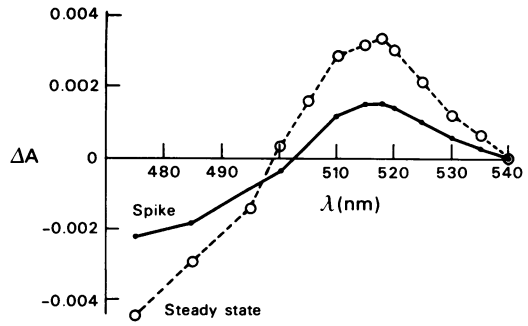

FIG. 2. Light *minus* dark difference spectrum of the absorbance change of chloroplasts, induced by red actinic light. pH 6.5. ●—●, spike; O---O, steady-state. Conditions as for Fig. 1. Measurements were reproducible to ±5%.

There is a resemblance between the pH responses of the light-induced 90° scattering and the steady-state of the 518 nm absorbance change, whereas the spike is less dependent on pH at values about 6.0 (Fig. 4B). The similar effect of pH on the spike and the steady-state below pH 6 may be due to the inhibition of electron flow.

Treatment of chloroplasts with 0.1% glutaraldehyde at 0°C caused a rapid decrease in light-induced 90° scattering at 518 nm (Fig. 5A). The steady-state level of the 518 nm absorption change was also inhibited by glutaraldehyde treatment, but it was not as sensitive as 90° scattering because of the increasing contribution from the fast component (spike) at the steady-state. The amplitude of the spike was considerably more resistant to inhibition by glutaraldehyde. In this experiment, we have corrected the steady-state level for the fast component to give the amplitude of the slow component. Comparison of Fig. 5A and B shows that the slow component of the 518 nm absorbance change and light-induced 90° scattering at 518 nm have similar sensitivities to glutaraldehyde, with a 50% loss in 5 min. The glutaraldehyde-treated chloroplasts retained considerable activity for light-induced proton uptake, showing 80% of the control after 5 min and 50% after 30 min (cf. 22).

The wavelength dependence of 90° scattering by chloroplasts at pH 6.5 and pH 8.0 in the absence of actinic light is shown in Fig. 6. The scattering spectra exhibit sharp maxima at 690 nm, and broad maxima in the region of 510–520 nm. Similar spectra have been observed in earlier studies with *Chlorella* (cf. 23). A spectrum for the light-induced

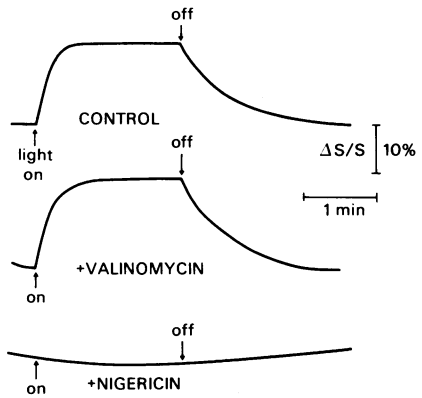

FIG. 3. Time course of light-induced 90° light scattering (ΔS/S) by chloroplasts at 518 nm. Assay medium as for Fig. 1. pH 6.5. Chlorophyll concentration, 1.5 μg/ml. Red actinic light (Corning 2-64) of intensity 20 mW cm^{-2}. Concentration of valinomycin and nigericin was 0.3 μM.

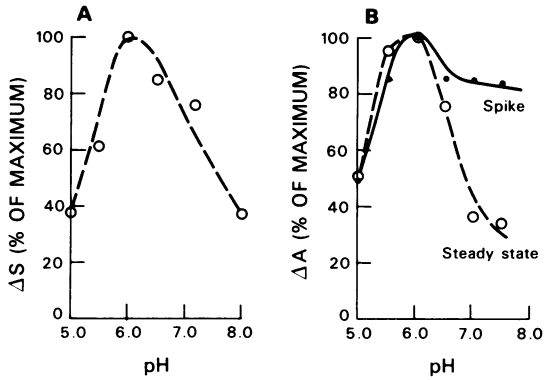

FIG. 4. Effect of pH on (A) light-induced 90° scattering at 518 nm, and (B) light-induced absorbance change at 518 nm; ●—●, spike; O--O, steady-state. All measurements were reproducible to ±5%.

change in scattering was determined in the blue-green region using strong red light as the actinic source (Fig. 7). The scattering amplitudes of the illuminated samples were normalized at 540 nm, to give a direct comparison between the light *minus* dark difference spectra of 90° scattering and the 518 nm absorbance changes (cf. Fig. 7 and Fig. 2).

Dissymmetry ratio

The dissymmetry ratio of light scattering depends on the size and shape of the scattering particles. Measurements of I_{45}/I_{135} were made at 518 nm, in the absence of actinic light. The measurements showed that the dissymmetry ratio is dependent on the chlorophyll concentration; it decreased from about 5 at 6 μg of chlorophyll/ml to about 2 at 0.75 μg of chlorophyll/ml.

DISCUSSION

The majority of studies on the light-induced absorbance change at 518 nm in isolated chloroplasts have been concerned with the "fast component," which is considered to be an indicator of changes in the electric field strength across the chloroplast thylakoid membrane (21). Larkum and Bonner (19) suggested that the "steady-state" signal for the ab-

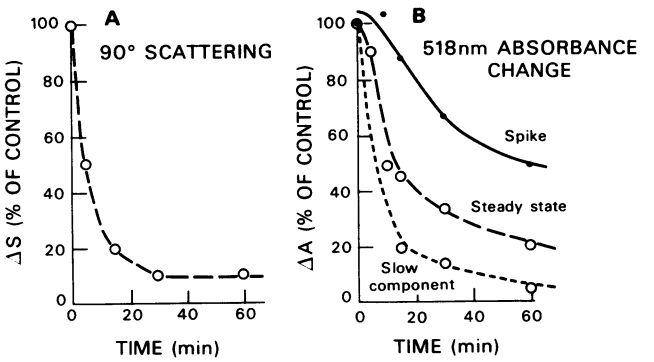

FIG. 5. Effect of 0.1% glutaraldehyde on (A) light-induced 90° scattering at 518 nm and (B) light-induced absorbance change at 518 nm. ●—●, spike; O--O, steady-state; O--O, slow component. See *text* for explanation of slow component. Glutaraldehyde at a final concentration of 0.1% was added to chloroplasts in assay medium at pH 7.5 at 0°C. Chlorophyll concentration was 1500 μg/ml. Aliquots were taken at intervals and the chloroplasts were assayed under conditions given in Figs. 1 and 3. pH 6.5. Measurements were reproducible to ±5%.

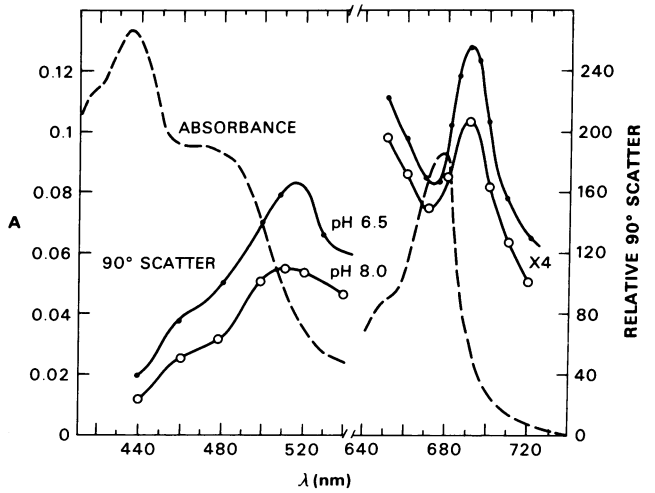

FIG. 6. Wavelength dependence of 90° scattering by chloroplasts in the absence of actinic light. ●—●, pH 6.5; ○—○, pH 8.0. Chlorophyll concentration, 1.5 μg/ml. The broken line shows an absorption spectrum of chloroplasts.

sorbance change at 518 nm in continuous actinic light is due to the fast and slow electrogenic mechanisms. The slower electrogenic mechanism is linked to photosynthetic electron transport and appears to be due to the movement of protons and other ions across the chloroplast thylakoid membrane (24). Larkum and Bonner (19) attributed the steady-state 518 nm absorbance change to the steady-state trans-membrane potential. Both the fast and steady-state components of the 518 nm absorbance change were considered to reflect an electric field across a carotenoid chromophore in the chloroplast thylakoid membrane, leading to an increase in absorbance at 518 nm (19, 21).

In the present work, we show that the amplitude of the 518 nm absorbance change at the steady-state increases strongly as the pH is lowered from 7.5 to 6.0. The steady-state signal consists of two components; one (the slow component) is abolished by nigericin and strongly inhibited by ammonium chloride, which suggests that it is related to light-induced proton movements. The steady-state signal which remains in the presence of nigericin is considered to reflect the remaining trans-membrane potential. The slow component was not particularly evident in earlier experiments (19) which were conducted at pH values above 7.

Our studies demonstrate a correlation between the slow component of the light-induced 518 nm absorbance change and 90° light scattering at 518 nm by chloroplasts. Both show similar responses to pH and to the ionophores, and both are inhibited with similar time courses when chloroplasts are treated with glutaraldehyde. We propose that the slow component of the absorbance increase is caused by the increase in scattering, rather than the effects of an electric field.

90° scattering changes of chloroplasts in the regions of 518 nm and 690 nm appear most likely to be a manifestation of the phenomenon known originally as anomalous dispersion, and later called selective dispersion (23, 25). Optical dispersion is due to the variation of the refractive index of the medium with the frequency of the electromagnetic radiation. With light-absorbing materials (chromophores), there is a fluctuation of the refractive index near the absorption peak. At wavelengths greater than the peak absorption, but within the absorption band, the refractive index increases slightly and at wavelengths below the peak absorption there is a slight decrease in refractive index (25). If the difference in refractive index between the chromophore and its surrounding medium is small, then a small change in the refractive index either of the chromophore or the medium will cause a significant change in the amount of light scattered from the chromophore.

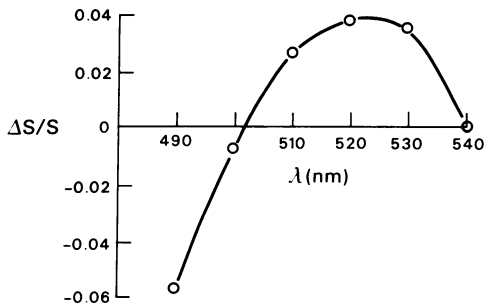

FIG. 7. Light *minus* dark difference spectrum of 90° scattering at 518 nm. pH 6.5. Chloroplasts were illuminated with red actinic light of intensity 20 mW cm^{-2}. Scattering amplitudes of the illuminated and nonilluminated samples were normalized at 540 nm. Chlorophyll concentration, 1.5 μg/ml.

The peaks in the 90° scattering spectra of chloroplasts (Fig. 6) are within the absorption bands of the photosynthetic pigments and lie on the high wavelength side of the absorption maxima. The positions and sharpness of the scattering peaks are consistent with the view that the enhanced scattering is caused by selective dispersion in the neighborhood of the absorption bands of the chromophores (23, 26). It seems likely that the pigments responsible for the selective dispersion are chlorophyll *a* in the red region (cf. 26) and one or more carotenoids in the blue-green region.

For spherical particles with small diameters compared with the wavelength of light ($d < \lambda/10$) light scattering is inversely proportional to λ^4 and the dissymmetry (I_{45}/I_{135}) is unity (25). Large particles of the size of chloroplasts scatter predominantly in the forward direction (Mie scattering) (27) and with a small angular profile (cf. 28–30). However, with chloroplasts we must also consider scattering from structures within the chloroplast, particularly the grana thylakoids (0.3 μm in diameter) where the pigments are predominantly localized, and particles within the thylakoid membranes.

Scattering from small particles within the thylakoid membranes would be expected to show a dissymmetry ratio close to unity for two reasons: (*i*) the particles are small compared with the wavelength of light and (*ii*) multiple scattering within the thylakoid membrane is likely. We propose that the decrease in the dissymmetry ratio at 518 nm with decreasing chloroplast concentration reflects a lower relative contribution of multiple scattering from whole chloroplasts, and a higher contribution from small chromophore-containing particles within the thylakoid membrane or from grana.

The effect of the ionophores indicates that light-induced changes in scattering at 518 nm are related to the proton gradient which is established across the chloroplast thylakoid membrane on illumination (13, 24, 31). Deamer *et al.* (14) showed a good correlation between the kinetics of light-induced proton uptake (ΔH^+) and the scattering change at 546 nm. We need to distinguish between the light-induced proton gradient across the thylakoid membrane (ΔpH), and the light-induced proton uptake (ΔH^+) which is dependent on the buffering capacities of the intra-thylakoid space and

the thylakoid membrane. ΔpH is greater if the external medium is buffered at pH 8.0, rather than at pH 6.5 (31), whereas ΔH^+ is maximal at an external pH of 6.0 (14). The light-dependent change in selective dispersion at 518 nm is maximal at pH 6.0 and it seems to correlate well with the extent of proton uptake, rather than with the magnitude of the proton gradient across the thylakoid membrane.

We propose that the increase in 90° selective scattering at 518 nm, induced either by light or in the dark by lowering the pH of the medium is due largely to a change in the refractive index difference between chromophore-containing particles and their immediate environment in the thylakoid membrane. The evidence in favor of pigment–protein complexes in the thylakoid membrane was reviewed recently (32). The change in the refractive index difference between the chromophore particles and their surroundings may result from proton uptake by the thylakoids from the medium. But protonation is apparently not sufficient to account for the light-induced or pH-induced change in selective scattering and the light-induced slow component of the 518 nm absorbance increase. These changes are very sensitive to treatment of chloroplasts with glutaraldehyde, whereas glutaraldehyde-treated chloroplasts retain considerable capacity for proton uptake. It is suggested that light-dependent increases in selective scattering and the slow component of the 518 nm absorbance will be observed only when some conformational change(s) of the chloroplast thylakoid membrane follow the proton uptake. The conformational changes may cause a reorientation of the carotenoid and chlorophyll–protein complexes in the lipoprotein matrix and bring the chromophore-containing particles into a region of lower refractive index. Glutaraldehyde is considered to inhibit the conformational change(s) by crosslinking regions of the membrane. Photophosphorylation is also very sensitive to glutaraldehyde fixation (33). The concentration of glutaraldehyde in our experiments was low compared to that used previously for the fixation of leaf tissue and isolated chloroplasts (22). The monitoring of scattering changes apparently is a sensitive method for detecting alterations in the thylakoid membrane induced by low concentrations of glutaraldehyde.

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1. Packer, L. (1962) *Biochem. Biophys. Res. Commun.* **9**, 355–360.
2. Itoh, M., Izawa, S. & Shibata, K. (1963) *Biochim. Biophys. Acta* **66**, 319–327.
3. Packer, L. & Deamer, D. W. (1967) in *Photophysiology*, ed. Giese, A. C. (Academic Press, New York), Vol. 3, pp. 91–122.
4. Murakami, S. & Packer, L. (1970) *J. Cell Biol.* **47**, 332–351.
5. Packer, L. & Siegenthaler, P. A.(1966) *Int. Rev. Cytol.* **20**, 97–121.
6. Crofts, A. R., Deamer, D. W. & Packer, L. (1967) *Biochim. Biophys. Acta* **131**, 97–118.
7. Mukohata, Y. (1966) *Annu. Rep. Biol. Works Fac. Sci. Osaka Univ.* **14**, 121–134.
8. Verkhoturov, V. N. & Tulbu, T. V. (1973) *Biofizika* **18**, 1052–1057.
9. Kushida, H., Itoh, M., Izawa, S. & Shibata, K. (1964) *Biochim. Biophys. Acta* **79**, 201–203.
10. Nobel, P. S. (1968) *Plant Physiol.* **43**, 781–787.
11. Heber, U. (1969) *Biochim. Biophys. Acta* **180**, 302–319.
12. Dilley, R. A. & Vernon, L. P. (1964) *Biochemistry* **3**, 817–824.
13. Jagendorf, A. T. & Hind, G. (1963) *"Photosynthetic mechanisms of green plants," Nat. Acad. Sci.-Nat. Res. Counc. Publ.* **1145**, 599–610.
14. Deamer, D. W., Crofts, A. R. & Packer, L. (1967) *Biochim. Biophys. Acta* **131**, 81–96.
15. Reeves, S. G. & Hall, D. O. (1973) *Biochim. Biophys. Acta* **314**, 66–78.
16. Heber, U. (1973) *Biochim. Biophys. Acta* **305**, 140–152.
17. West, K. R. & Wiskich, J. T. (1968) *Biochem. J.* **109**, 527–532.
18. Boardman, N. K. & Thorne, S. W. (1968) *Biochim. Biophys. Acta* **153**, 448–458.
19. Larkum, A. W. D. & Bonner, W. D. (1972) *Biochim. Biophys. Acta* **256**, 396–408.
20. Larkum, A. W. D. & Boardman, N. K. (1974) *FEBS Lett.* **40**, 229–232.
21. Witt, H. T. (1971) *Quart. Rev. Biophys.* **4**, 365–477.
22. Packer, L., Allen, J. M. & Starks, M. (1968) *Arch. Biochem. Biophys.* **128**, 142–152.
23. Latimer, P. & Rabinowitch, E. (1959) *Arch. Biochem. Biophys.* **84**, 428–441.
24. Mitchell, P. (1966) *Biol. Rev. Cambridge Philos. Soc.* **41**, 445–502.
25. Partington, J. R. (1953) "An advanced treatise on physical chemistry," *Physico-Chemical Optics* (Longmans, Green and Co., London), Vol. 4.
26. Latimer, P. (1959) *Plant Physiol.* **34**, 193–199.
27. Mie, G. (1908) Ann. Physik. **25**, 377–445.
28. Bryant, F. D., Seiber, B. A. & Latimer, P. (1969) *Arch. Biochem. Biophys.* **135**, 97–108.
29. Gregory, R. P. F. & Raps, S. (1974) *Biochem. J.* **142**, 193–201.
30. Koch, A. L. (1961) *Biochim. Biophys. Acta* **51**, 429–441.
31. Rottenberg, H., Grunwald, T. & Avron, M. (1972) *Eur. J. Biochem.* **25**, 54–63.
32. Thornber, J. P. (1975) *Annu. Rev. Plant Physiol.* **26**, 127–158.
33. West, J. & Packer, L. (1970) *Bioenergetics* **1**, 405–412.