ELECTRON TRANSPORT REACTIONS IN GRANA PREPARATIONS FROM SPINACH CHLOROPLASTS

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ABSTRACT Fraction 2 (grana-stack) particles prepared with the French press showed absorbance changes, at room temperature and with sodium ascorbate and methylviologen, that were produced by the oxidation of cytochrome *b*-559. This oxidation was inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and sensitized by system II of photosynthesis. The oxidation is too slow to account for the rates of the Hill reaction that have been observed with nicotinamide-adenine dinucleotide phosphate ($NADP^+$). It appears that this cytochrome is not functioning in the main pathway of electron transport. In the presence of 2,3,5,6-tetramethyl-*p*-phenylenediamine (DAD) and ascorbate, light-induced oxidation of cytochrome *f* took place within 3 msec (or faster) in the grana-stack particles. Treatment with the detergent Triton X-100 disrupted this rapid cytochrome *f* oxidation as well as the oxidation of cytochrome *b*-559. Subsequent plastocyanin addition did not restore the rapid oxidation of cytochrome *f* (nor of cytochrome *b*-559) but only slow changes of cytochrome *f*. In view of the fact that these particles contain almost no plastocyanin, it is unlikely that plastocyanin functions in electron transport between cytochrome *f* and P-700 in the particles derived from the grana-stack regions of the chloroplast.

INTRODUCTION

A method has been developed by Michel and Michel-Wolwertz (1967, 1969) for preparing two kinds of chloroplast particles by a procedure that does not require the use of detergents or sonic treatment but rather employs the French pressure cell to disrupt chloroplasts. Centrifugation of the resulting fragments on a sucrose density gradient yields two types of particles: light particles (fraction 1) exhibiting system I activity and heavy particles (fraction 2) having both system I and system II activities.

In their studies of the ultrastructure of chloroplasts and separated particles, Jacobi and Lehmann (1968) as well as Sane et al. (1970) concluded that small particles having only system I activity come from stroma lamellae and that large particles having both system I and II activities come from grana-stack regions of the chloroplast.

We have recently studied the oxidation-reduction reactions of P-700 and cytochrome *f* as well as the cytochrome b_6 reactions in the small fraction 1 particles. It appeared from this study that electron transport remained more intact in these particles than in particles prepared with detergent or sonic treatment.

Our most recent study (Murata and Fork, 1971) has shown that both types of particles prepared by the Michel procedure contain almost no plastocyanin. In the case of fraction 1 (stroma) particles there was about 1 plastocyanin to 3500 chlorophylls (on a molar basis) and in the fraction 2 (grana-stack) particles about 1 plastocyanin to 7000 chlorophylls. The fraction 1 particles that contained almost no plastocyanin nevertheless could still perform a rapid, light-induced, cytochrome *f* to P-700 electron transfer similar to that seen in whole chloroplasts. Since grana-stack particles are also devoid of plastocyanin, it was of interest to see if they also could perform rapid cytochrome *f* to P-700 electron transport reactions.

Sane et al. (1970) found that cytochrome *b*-559 was absent from the small fraction 1 particles but present in grana-stack particles. The likelihood existed that this cytochrome could be seen functioning in the grana-stack particles and, if so, it would be possible to study which photochemical system is responsible for the photooxidation of this cytochrome. Such a determination is needed in view of conflicting reports that have appeared (Levine et al., 1966; Cramer and Butler, 1967; Hind, 1968 *a*; Ben Hayyim and Avron, 1970; Knaff and Arnon, 1969; Boardman et al., 1971; Floyd et al., 1971).

MATERIALS AND METHODS

Chloroplasts were prepared by grinding spinach leaves in a Waring Blendor (Waring Products Div., New Hartford, Conn.) for about 10 sec in a solution containing 400 mM sucrose, 50 mM phosphate buffer, pH 7.7, and 10 mM NaCl. The resulting slurry was filtered through one layer of "Miracloth" (Calbiochem, San Diego, Calif.) and centrifuged at 1000 *g* for 5 min. The precipitate was resuspended in a solution containing 150 mM KCl and 50 mM Tricine-KOH buffer, pH 7.8, and centrifuged at 270 *g* for a few seconds to sediment whole cells and large fragments. The supernatant containing chloroplasts was passed three times through the French pressure cell at 12,500 psi (800 kg/cm^2). The resulting suspension of disrupted chloroplasts was centrifuged at 1000 g for 5 min to remove unbroken chloroplasts and the resulting supernatant was then centrifuged at 10,000 *g* for 15 min. The sediment containing the grana-stack particles was resuspended with the aid of a tissue homogenizer in the KCl-Tricine buffer described above and centrifuged for 5 min at 1000 *g* to remove large or aggregated particles. The supernatant was used as the grana-stack preparation. Plastocyanin obtained from *Atriplex* leaves by a procedure similar to that used by Katoh et al. (1962) was kindly supplied by Dr. J. S. Brown.

Light-induced changes of absorbance were measured as described previously (Fork and Murata, 1971). The measuring beam had a half-bandwidth of 2 nm for all experiments. For measurements in the blue and green regions of the spectrum, the photomultiplier (EMI 9558B, Varian/EMI, Gencom Div., Plainview, N.Y.) was protected by using two Corning filters 9782 (CS 4-96, Corning Glass Works, Corning, N.Y.) and a Calflex C filter. For action spectra measurements, the photomultiplier was covered by Corning 9782 and 5113 (CS 5-58) and a Calflex C filter, and was insensitive to the red actinic light that was obtained

by filtering light from a 650 w quartz-iodine lamp through 2.7 cm of water, a Calflex heat-reflecting filter (Balzers High Vacuum Corp., Santa Ana, Calif.), and 3 mm thick Schott RG5 colored glass filter (Fish-Schurman Corp., New Rochelle, N.Y.). This red actinic light had wavelengths extending from 650 to 750 nm and an intensity of 4×10^6 ergs cm^{-2} sec^{-1} and was used for all experiments except for action spectra measurements.[1]

For measurements of action spectra, pairs of Balzers interference filters were used to produce various monochromatic actinic wavelengths having half-bandwidths of about 9 nm. The far red background light (when used) was defined by using 3 mm of a Schott RG 10 filter plus a Calflex C filter, and had an intensity of about 1.1×10^4 ergs cm^{-2} sec^{-1} in a band extending from 700 to 750 nm.

A sample cuvette having a path length of 0.5 cm was used for all experiments. Air was used as the gas phase for all experiments.

RESULTS

Fig. 1 shows examples of the kinetics of absorbance changes measured at 420, 425, and 430 nm in grana-stack particles to which 10 μM methylviologen and 1 mM ascorbate were added. At 420 nm (trace *a*) a rapid decrease of absorbance occurs upon illumination. The steady level of the change during illumination is attained rapidly. The kinetics are different at 425 and 430 nm. Here rapid changes are produced upon illumination (as indicated in traces *b* and *c* of Fig. 1). These rapid changes are followed by a slower decrease that requires about 1 sec to reach an approximate steady state.

Traces *d*, *e*, and *f* of Fig. 1 show that the addition of 100 μM DCMU inhibits only the slow change at 425 and 430 nm, but not the rapid change. The rapid decrease at 420 nm is similarly not inhibited by DCMU. (A small amount of slow change persists at all three wavelengths after treatment with DCMU.)

Difference spectra are given in Fig. 2 A for the steady-state levels of the changes shown in traces *a*, *b*, and *c* of Fig. 1. Positive maxima occur at 412 and around 447 nm. A negative peak occurs in the blue around 427 nm and in the green at 559 nm. This spectrum suggests that the slow absorbance decrease is produced by oxidation of cytochrome *b*-559 in the grana-stack particles. The difference spectrum in Fig. 2 B shows that this oxidation of cytochrome *b*-559 is inhibited by DCMU. After treatment, maxima were seen around 403 and 448 nm, and a minimum occurred at 420 nm. A broad shoulder appeared around 430 nm and no measurable changes were seen in the green region around 550 nm.

The results with DCMU suggest that oxidation of cytochrome *b*-559 is mediated by system II rather than by system I. It was of interest, therefore, to compare the action spectrum for the oxidation of this cytochrome with the action spectrum for a known system I reaction such as that for the oxidation of P-700.

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[1] Murata and Brown (1971) measured the system II activity in these particles and found them to retain about 56% of the activity of the intact chloroplasts (using ferricyanide as the Hill oxidant). The particles used here were made under conditions identical with those used by Murata and Brown (1971).

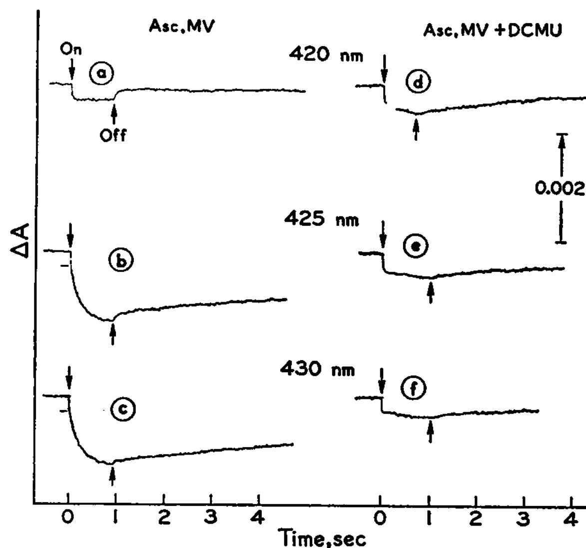

FIGURE 1

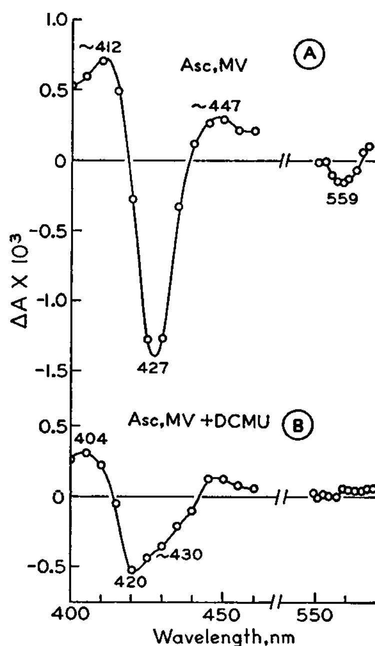

FIGURE 2

FIGURE 1 Light-induced changes of absorption at 420, 425, and 430 nm in grana-stack particles from spinach. The final concentrations of substances in the reaction mixture were: sodium ascorbate, 1 mM; methylviologen (MV), 10 μM; KCl, 131 mM; Tricine-KOH buffer (pH 7.8), 44 mM; chlorophyll concentration, 12.3 μg/ml; and, when added, DCMU, 100 μM. Arrows pointing downward and upward mark when the actinic light was turned on and off respectively.

FIGURE 2 Light minus dark difference spectra for grana-stack particles from spinach obtained using sec 1 exposures to red actinic light. The spectra were obtained using the same sample and contained the same additions as used for Fig. 1. The spectra were measured by taking the change that resulted after 1 sec of illumination (approximately the steady state).

Action Spectrum for Cytochrome b-559 Oxidation

The action spectrum for the rate of absorbance changes at 428 nm was measured for grana-stack particles to which 10 μM methylviologen and 1 mM ascorbate were added. In order to avoid confusion by rapid absorbance decreases produced by other compounds such as cytochrome *f* or P-700, a far red background light of sufficient intensity was chosen so that the rates of the absorbance changes produced at 428 nm were linear up to an intensity of about 5.1 nanoeinsteins cm^{-2} sec^{-1} at 683 nm. A similar relationship was found when actinic light at 673 nm was used. The action spectrum was determined using equal numbers of incident quanta (equivalent to 2.7 nanoeinsteins cm^{-2} sec^{-1}) and is given in Fig. 3. It can be seen that this spectrum is clearly a system II–type action spectrum since it has a peak around 673 nm, a broad shoulder in the 650 nm region, and rapid decline of activity in the near infrared region. The insert of Fig. 3 shows the difference spectrum

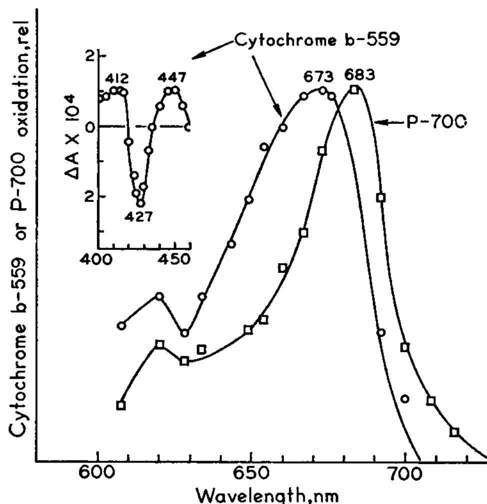

FIGURE 3 Action spectra for the oxidation of cytochrome *b*-559 and P-700 in grana-stack particles from spinach. The action spectrum for the oxidation of cytochrome *b*-559 was obtained with far red background light (described in the text) by measuring the rate of the slow absorbance decrease at 428 nm upon illumination. A dark interval of 2 min was used between exposures to actinic wavelengths. The insert shows the difference spectrum for absorbance changes produced by the same sample used for the action spectrum measurement. The reaction mixture contained: sodium ascorbate, 1 mM; methylviologen, 10 μM; KCl, 144 mM; Tricine buffer, 48 mM; the concentration of chlorophyll was 14.2 μg/ml. This concentration of chlorophyll in the 0.5 cm cuvette gave 53% absorption at 680 nm (measured in an integrating sphere). For the action spectrum for P-700 oxidation, the sample contained the substances listed above and, in addition, DAD and DCMU at a final concentration of 50 and 100 μM respectively. Measurements were made of the rate of absorbance changes at 430 nm upon illumination (without background light).

measured with the same far red background light and using the same sample as used for the action spectrum measurement. This difference spectrum confirms that the action spectrum measured at 428 nm is produced by cytochrome *b*-559.

In order to compare the action spectrum for cytochrome *b*-559 oxidation with an action spectrum for system I, the grana-stack fragments containing 10 μM methylviologen and 1 mM ascorbate were treated with 100 μM DCMU and 50 μM DAD. Under this condition all the cytochrome *b*-559 changes are eliminated and only changes caused by cytochrome *f* and P-700 can be seen. Absorbance changes at 430 nm were taken as those produced by P-700 since the contribution of cytochrome *f* to absorbance changes at this wavelength are small (Fork and Murata, 1971). This system I action spectrum is also shown in Fig. 3. It has a peak at 683 nm, low activity in the 650 nm region, and activity extending to considerably longer wavelengths than the action spectrum for cytochrome *b*-559 oxidation. The difference spectrum (measured on a different sample) for the changes produced in the presence of methylviologen, ascorbate, DAD, and DCMU with 683 nm actinic light was

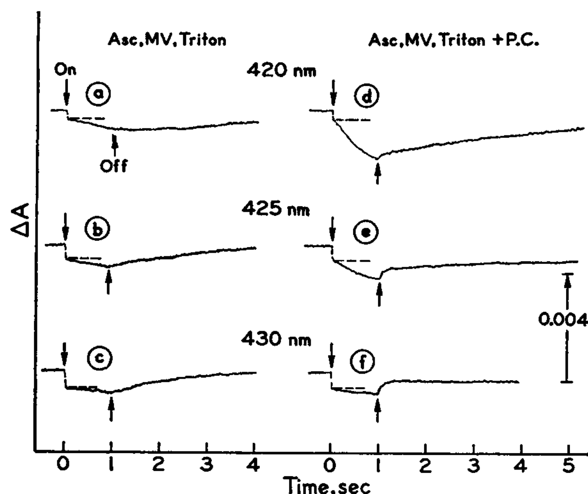

FIGURE 4 The effect of Triton X-100 and of plastocyanin on absorbance changes at 420, 425, and 430 nm in grana-stack particles from spinach. The sample was illuminated with the same actinic light as for Fig. 1. The final concentrations of substances in the reaction mixture (used to obtain traces *a*, *b*, and *c*) were: sodium ascorbate, 0.9 mM; methylviologen, 9.2 μM; Triton X-100, 0.13%; KCl, 120 μM; Tricine buffer, 40 μM. Plastocyanin (P.C.) (0.4 μM, final concentration) was added to the reaction mixture before obtaining traces *d*, *e*, and *f*. The chlorophyll concentration was 23.5 μg/ml.

found to be like those shown later in Figs. 5 A and B or 6 A and C and appears to be produced by changes in the Soret band of P-700.

The Effects of Detergent on Cytochrome b-559 Changes

The effects of Triton X-100 upon the kinetics of absorbance changes at 420, 425, and 430 nm in the presence of 9.2 μM methylviologen and 0.9 mM ascorbate are shown in Fig. 4. No significant slow changes remain at these wavelengths after Triton (0.13%) treatment. The pattern of the rapid absorbance decrease is changed also. After treatment, the rapid decrease at 420 nm is only about half of that produced at 430 nm. Before Triton treatment, the rapid change is larger at 420 than at 430 nm (cf. traces *d* and *f* of Fig. 1). The difference spectrum for the rapid absorbance decrease seen in the presence of Triton X-100 is given in Fig. 5 A (top curve). This spectrum indicates that P-700 alone is producing the rapid absorbance changes in grana particles after Triton treatment. A similar effect of Triton on the rapid cytochrome *f* absorption change was also seen in system I particles prepared by means of the French pressure cell (Fork and Murata, 1971).

The addition of 0.4 μM plastocyanin to grana-stack particles containing methylviologen, ascorbate, and Triton restored a slow absorbance change at 420 and, to a lesser extent, at 425 nm (Fig. 4, traces *d* and *e*). Fig. 4 also shows that the rapid absorbance change is unaffected by plastocyanin addition. Thus the difference spectrum for the rapid change seen after adding plastocyanin (Fig. 5 B, middle

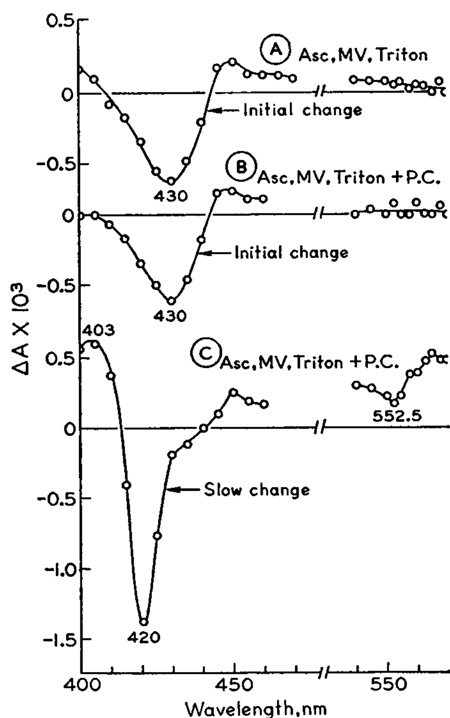

FIGURE 5 Light minus dark difference spectra obtained using the same sample of grana-stack particles as used for Fig. 4. Spectrum A was measured by taking the initial rapid deflection seen upon illumination (as indicated on the traces of Fig. 4) and the substances present, in addition to KCl and Tricine, were ascorbate (Asc), methylviologen, and Triton X-100. The spectra labeled B and C were obtained for the rapid and slow changes respectively after addition of plastocyanin.

curve) is identical with that before adding plastocyanin (Fig. 5 A, top curve). The difference spectrum for the slow change is shown in Fig. 5 C (bottom curve). This spectrum was constructed by taking the absorbance decrease occurring after the initial rapid decrease (below the dotted lines in traces *d*, *e*, and *f* of Fig. 4). This difference spectrum (Fig. 5 C) has maxima around 403 and 450 nm and minima at 420 and 552.5 nm, and demonstrates clearly that plastocyanin restores slow oxidation of cytochrome *f* in detergent-treated grana-stack particles.

An interesting effect was seen upon adding DAD to the same grana-stack preparation used for Fig. 5 that contained methylviologen, ascorbate, Triton, and plastocyanin. Fig. 6 B shows that when both 44 μM DAD and 0.4 μM plastocyanin were present, the spectrum for the slow change had a maximum near 412 nm and minima at 427 and 559 nm, indicating that under this condition cytochrome *b*-559 and not cytochrome *f* was oxidized. By contrast, the spectrum for the rapid decrease (Fig. 6 A) was unaffected by subsequent plastocyanin addition and demonstrates that the rapid changes were still produced by P-700 oxidation. Further addition of 87

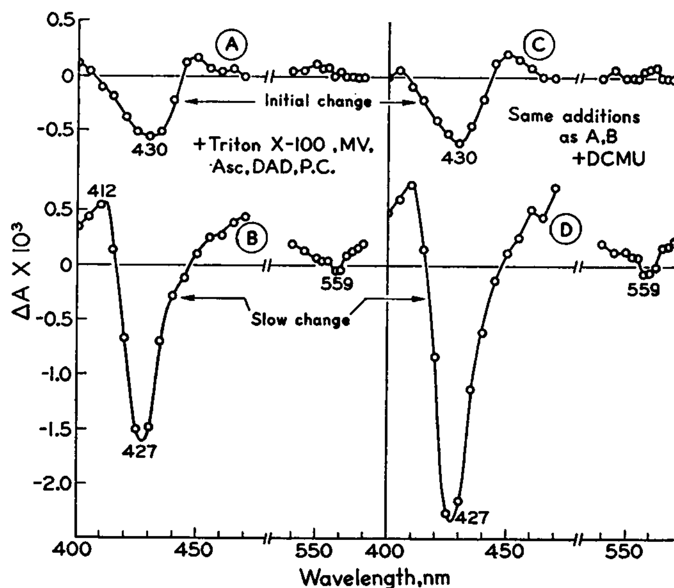

FIGURE 6 Light minus dark difference spectra obtained using the same sample of grana-stack particles as used for Figs. 4 and 5. The spectra labeled A and B were obtained after adding DAD to a final concentration of 44 μM in addition to the substances present as described for Fig. 5. For spectra shown in C and D, subsequent addition of DCMU (87 μM) was made.

μM DCMU to this preparation (Fig. 6 D) caused an increase of the slow light-induced cytochrome *b*-559 oxidation and no effect on the difference spectrum for the initial change produced by P-700 (Fig. 6 C).

The addition of DAD to grana-stack particles treated with Triton and containing ascorbate and methylviologen (not shown as a figure) did not restore the slow changes. The difference spectrum for rapid changes seen with these additions suggested P-700 participation and resembled those shown in Figs. 5 A and B, 6 A and C.

Absorbance Changes in Grana-Stack Particles in the Absence of Triton

Fig. 7 shows examples of absorbance changes at 420, 425, and 430 nm in the grana-stack particles in the absence of Triton but in the presence of 38.5 μM DAD, 1.0 μM ascorbate, 10 μM methylviologen, and 96 μM DCMU (traces *a*, *b*, *c*) and after subsequent addition of 0.4 μM plastocyanin (traces *d*, *e*, and *f*). It can be seen that upon illumination, a rapid decrease of absorbance takes place that is not followed by further, slower absorbance changes such as seen after Triton treatment. This is true whether plastocyanin is present or not. The difference spectra for these rapid changes, before and after plastocyanin addition, are given in Fig. 8. The maxima at 403 nm and minima at 420 and 552 nm suggest the light-induced oxidation of cyto-

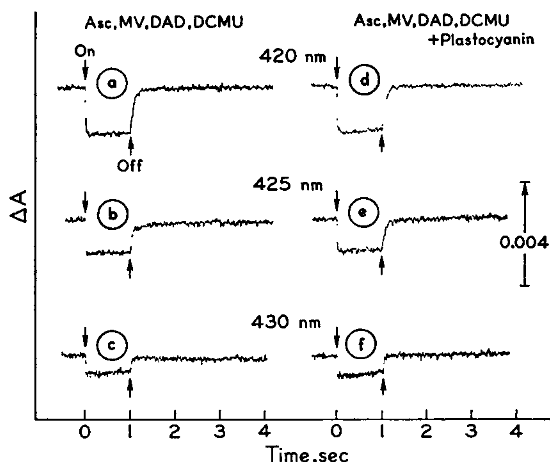

FIGURE 7 Light-induced changes of absorption at 420, 425, and 430 nm in grana-stack particles from spinach. The final concentrations of substances in the reaction mixture used for traces *a*, *b*, and *c* were: sodium ascorbate, 1.0 mM; DAD, 38.5 μM, methylviologen, 10 μM; DCMU, 96 μM; KCl, 44.5 mM; Tricine buffer, 133 mM; and, for traces *d*, *e*, and *f*, plastocyanin was subsequently added to a final concentration of 0.4 μM. Chlorophyll concentration, 22.8 μg/ml.

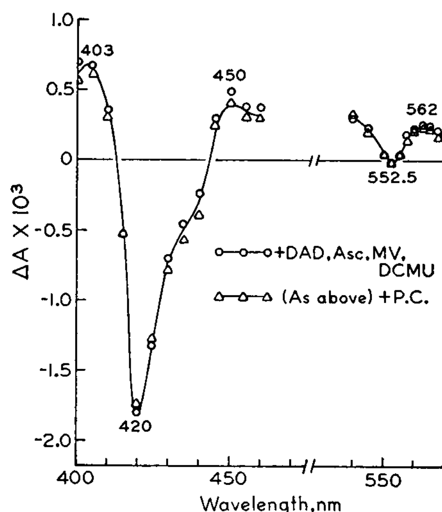

FIGURE 8 Light minus dark difference spectra for the rapid changes of absorption in grana-stack particles obtained from spinach using the same samples described for Fig. 7. Circles: sodium ascorbate, DAD, methylviologen, and DCMU; open squares, plastocyanin added in addition.

chrome *f*. The maximum at 562 nm may indicate reduction of cytochrome *b*-562 as may the shoulder around 435 nm. This shoulder may be also produced, in part, by P-700.

DISCUSSION

The first observations of light-induced oxidation of cytochrome *b*-559 by system II were seen only at 77°K (Knaff and Arnon, 1969; Boardman et al., 1971; Floyd et al., 1971). Recently, Knaff and Arnon (1971) reported a slow oxidation of cytochrome *b*-559 at room temperature and at pH 10 mediated by system II that had a half-time of about 3.5 sec.

It is clear from the results reported here that in grana-stack particles cytochrome *b*-559 is oxidized at a physiological pH (7.8) and at room temperature by system II because this oxidation was abolished by the system II inhibitor DCMU and the action spectrum for the oxidation was typical of that produced by a system II reaction. Chance et al. (1969) have also seen oxidation of cytochrome *b*-559 at low temperature that appears to be sensitized by system II, although at that time it was suggested that the changes they measured were produced by cytochrome *f*.

In the grana-stack particles not treated with Triton, the oxidation of cytochrome *b*-559 (unlike cytochrome *f* oxidation) is a relatively *slow* reaction. In Fig. 1 *b*, for example, it was seen that the half-time for this change was around 0.15 sec. The oxidation-reduction rates of cytochrome *b*-559 are too slow to explain the rates of the Hill reaction that can be seen with $NADP^+$. In view of these results, the scheme proposed by Knaff and Aron (1971) where cytochrome *b*-559 functions in the main pathway of electron transport does not seem plausible. It would be more reasonable to assume that this cytochrome functions in a side pathway connected to system II. A similar conclusion was reached by Boardman et al. (1971) based upon experiments on the light-induced oxidation of this cytochrome at low temperature. Boardman et al. (1971) found no evidence that more than one type of cytochrome *b*-559 was undergoing light-induced oxidation at low temperature.

Also, in the scheme proposed by Knaff and Arnon (1971), plastocyanin is an intermediate in the photooxidation of cytochrome *b*-559 by system II ("IIa"). Again, these results do not support this scheme because oxidation of cytochrome *b*-559 by grana-stack particles was observed without added plastocyanin. This electron carrier is not bound in the grana-stack particles because a recent analysis by Murata and Fork (1971) has shown them to contain almost no plastocyanin (about 1 plastocyanin to 7000 chlorophylls on a molar basis.)

Other studies on cytochrome *b*-559 have suggested that it functions between the two light reactions and is oxidized by system I and reduced by system II (Levine et al., 1966; Cramer and Butler, 1967; Hind, 1968 *a*; Ben Hayyim and Avron, 1970). In all these studies, the half-times for cytochrome *b*-559 oxidation produced by system I were even slower than those seen here produced by system II. Perhaps the reactions of this cytochrome are affected by the manner in which chloroplasts or particles are prepared. For example, after Triton X-100 disruption of grana-stack particles, system I apparently oxidized cytochrome *b*-559 and system II reduced it because DCMU produced a stimulation of the oxidation rather than the inhibition

that was seen before Triton treatment (compare Figs. 6 B and D). This conclusion, however, must be confirmed by further action spectra measurements for cytochrome *b*-559 oxidation under these conditions.

Grana-stack particles before treatment with Triton show rapid cytochrome *f* changes. In these experiments the rise time was 3 msec or faster and was limited by the rise time of the measuring system used. Similar fast cytochrome reactions were found in fraction 1 particles isolated from chloroplasts fragmented in the French pressure cell (Fork and Murata, 1971). In the latter particles, the rise time was found to be within 1 msec when a faster measuring system was used. Thus both types of particles have rapid cytochrome changes. Hildreth (1968) measured half-times for cytochrome *f* oxidation to range from 0.3 to 9 msec in spinach chloroplasts excited with a 30 nsec flash from a *Q*-switched ruby laser. It appears that the preparation of grana-stack particles (as with fraction 1 particles) by means of the French press leaves most of the native electron transport system of system I intact. The presence of detergent changes the particles and disrupts these rapid cytochrome *f* reactions.

As mentioned earlier, the grana-stack particles contain almost no plastocyanin and yet Figs. 7 and 8 demonstrated that added plastocyanin had no effect on any of the rapid absorbance changes investigated. A very different result was obtained with grana-stack particles treated with detergent. The only rapid absorbance changes seen after this treatment were produced by P-700. Subsequent addition of plastocyanin had a significant effect only on the restoration of slow cytochrome *f* oxidation. This slow oxidation of cytochrome *f* restored by added plastocyanin had a half-time of several tenths of a second. A similar effect in restoring only slow cytochrome *f* oxidation by added plastocyanin was found in Triton-treated grana-stack particles in the presence of DAD and ascorbate (Fork and Jacobi, 1969). Thus, conclusions about the site of plastocyanin in the electron transport chain based upon the ability of plastocyanin to restore cytochrome *f* oxidation are not warranted, since these measurements were all made with equipment that can only detect absorbance changes occurring after several tenths of a second (Gorman and Levine, 1966; Hind, 1968 *b*; Avron and Shneyour, 1971). No demonstration has yet been made of the restoration by added plastocyanin of a *rapid* cytochrome *f* oxidation such as that discussed earlier.

In view of the absence of plastocyanin in the grana-stack particles and of their rapid cytochrome *f* oxidation (before Triton treatment), it is unreasonable to place plastocyanin as an electron transport component functioning between P-700 and cytochrome *f*. An alternative to this formulation would be to place plastocyanin before cytochrome *f* (Fork and Urbach, 1965; Avron and Chance, 1966) or on a pathway independent of, but parallel to, cytochrome *f* (Kok, et al., 1964).

It was interesting to note that the slow oxidation of cytochrome *b*-559, after Triton treatment, could only be seen after both plastocyanin and DAD were added. The addition of DAD alone did not restore any slow changes. As has been discussed

earlier, the addition of plastocyanin to detergent-treated particles restored only the slow cytochrome *f* changes, but when both DAD and plastocyanin were present the slow oxidation of cytochrome *b*-559 was seen. Added plastocyanin is very effective in reacting with P-700 in detergent-treated fraction 1 particles from spinach (Fork and Murata, 1971). It is reasonable to suppose a similar situation exists for grana-stack particles treated with detergent. Added plastocyanin also appears to react with the cytochrome *f* (but not with cytochrome *b*-559) that is dislodged from its functional site by detergent action. DAD may act as a bridge to connect cytochrome *b*-559 via cytochrome *f* to P-700.

The P-700 seen in the grana-stack particles cannot be considered a "contaminant" because repeated passage through the French press (five times) did not remove it. The light minus dark difference spectrum for the particles fragmented many times was like that reported (Fork and Murata, 1971) for fraction 1 particles and had minima at 682 and 701 nm (Fork and Jacobi, unpublished results). Thus the P-700 in the grana-stack particles seems to be firmly bound in them and it seems reasonable to assume that both systems I and II function in grana regions of chloroplasts.

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