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THE 520-nm LIGHT-INDUCED ABSORBANCE CHANGE IN PHOTOSYNTHETIC MUTANTS OF SCENEDESMUS

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SUMMARY

The 520-nm light-induced absorbance change was examined in intact cells and chloroplast preparations of the wild type strain and six photosynthetic mutants of *Scenedesmus obliquus*, strain D_3. The two phases of the absorbance change observed under aerobic conditions were separated by criteria other than their kinetic differences. In intact cells the rapid phase did not exhibit a requirement for an electron donor while the slow phase did. The slow phase possessed a requirement for System-II activity only in the absence of an electron donating system alternate to water. All six mutants (three System II, two electron transport and one System I) showed only the first phase of the absorbance change *in vivo*. We, therefore, postulated that both phases of the 520-nm absorbance change (*in vivo*) are directly related to System I and that System II is only indirectly involved. Discrepancies between results obtained with chloroplast preparations and with intact cells are also discussed.

INTRODUCTION

Application of spectrophotometric techniques to the study of the electron transport pathway in photosynthesis has revealed many details regarding the identification and sequence of components of that pathway. However, the significance of one of the largest light-induced absorbance changes, with a broad peak near 520 nm, remains unknown even though it was among the first to be described[1].

Both light reactions of photosynthesis have been found to be responsible for the complex nature of the 520-nm light-induced absorbance change and three hypotheses have been developed to explain the involvement of the two photosystems[2-4]. Recent evidence has indicated the presence of two compounds, at least in higher plants, which are responsible for the 520-nm change[5]. Several chloroplast components have been proposed as the agents responsible for all or part of this absorbance change, including chlorophyll *b* and a specific form of chlorophyll *a* (ref. 2), a carotenoid[6], β-carotene[7] an unidentified semi-reduced compound[3], and a chlorophyll–carotenoid complex[8].

Abbreviations: DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; CCCP, *m*-chlorocyanocarbonylphenylhydrazone.

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We have examined the characteristics of the 520-nm change in several photosynthetic mutants of *Scenedesmus obliquus*, strain D_3, in an attempt to evaluate the relationship of this absorbance change to photosynthetic activity as well as to examine the various hypotheses which have already been proposed. Properties of the mutants used in this study have been described elsewhere[9]. Two of the mutants (26 and 50) are defective in the electron-transport chain connecting the two light reaction centers of photosynthesis, three (11, 40, and a′) are defective in the oxygen evolving light reaction (System II), and a sixth mutant (8) is defective in System I. This last mutant, which lacks the chloroplast component designated P700 (refs. 11–13) is the only mutant whose defect has been specifically identified. Current unpublished data suggest that 26 and 50 are deficient in an F-type cytochrome.

METHODS

Algal culture and chloroplast preparation

The methods involved in the maintenance, culture, and harvesting of the various algal strains have previously been described[10]. The data reported here were obtained with 5-day-old cultures which had a packed cell volume of approx. 10 μl/ml.

Chloroplasts were isolated as described earlier for chloroplast photoreduction studies[10]. Chlorophyll was measured using the absorption coefficients given by Mackinney for chlorophylls *a* and *b* in methanol (see ref. 14).

Single-beam spectrophotometer

A single-beam spectrophotometer for absorbance measurements with light scattering samples was constructed following the basic design of DE KOUCHKOVSKY AND FORK[15]. A 525-nm Schott narrow band pass interference filter isolated the measuring light. Three red cut-off filters (2 Corning 2-58 and 1 Corning 2-59), an infrared absorbing filter, and a 5-cm water bath isolated the actinic light. A second Schott 525 nm interference filter, a blue Corning glass filter (4-71), and an infrared absorbing filter were placed over the surface of the photomultiplier (RCA 6217) to prevent the actinic light from reaching the photocathode. The sample holder was then placed directly over these filters. The measuring light intensity was approx. 2500 $\text{ergs}\cdot\text{cm}^{-2}\cdot\text{sec}^{-1}$; the actinic intensity was approx. $6\cdot10^4$ $\text{ergs}\cdot\text{cm}^{-2}\cdot\text{sec}^{-1}$.

The output of the photomultiplier was displayed either on an oscilloscope (Hewlett-Packard 140 A) or a high-speed strip chart recorder (BLH Meterite, BSA 250 with PR 301B amplifier) after passing through a filter with a time constant of less than 5 msec.

Two sample holders were employed, one with a cross sectional area of 1 cm^2 and one of 10 cm^2. The former cuvette was used with chloroplast preparations and lacked facilities for gas phase control. The latter cuvette was used for all *in vivo* measurements and provided control of both temperature and gas phase. The measuring light passed through the sample in a vertical direction to minimize the effects of settling of the algal cells.

Double-beam spectrophotometer

A double-beam spectrophotometer utilizing a mechanical light chopper and a phased lock-in amplifier (Princeton Applied Research, model JB-4) was constructed utilizing many of the components of the single-beam instrument described above. The increased gain (approx. 104) and stability provided by the lock-in amplifier permitted the use of much lower intensity measuring light (estimated to be about 100 ergs·cm^{-2}·sec^{-1}). A time constant of 0.1 sec was used. The output of the amplifier was then displayed on either the oscilloscope or a strip-chart recorder (Photovolt Varicord, model 43). The use of such a relatively long time constant precluded the type of kinetic measurements made with the single-beam spectrophotometer.

The measuring light was separated into two paths by a partially silvered mirror. A 571-nm narrow band pass interference filter was used to isolate the reference wavelength, which was chosen because of the absence of a known light-induced absorbance change at that wavelength. The measuring wavelength was obtained from a Bausch and Lomb high-intensity monochromator. The exit slit on the monochromator was maintained at 1 mm, while the entrance slit was varied as necessary to adjust the light intensity. The two wavelengths were then recombined by a second beam splitter and focused onto the sample after passing through the light chopper.

The actinic source was a 150 W high pressure xenon arc. Narrow band pass interference filters were used to isolate actinic light for relative quantum efficiency determinations. Appropriate Corning glass filters were placed over the surface of the photomultiplier to protect the photocathode from the actinic radiation.

The 10 cm^2 surface area sample holder was used for all measurements with the double-beam spectrophotometer. The double-beam instrument was used to obtain the difference spectra and to determine the relative quantum efficiencies for the 520-nm light-induced absorbance change.

RESULTS

Single-beam spectrophotometry (whole cells)

Examples of light-induced 520-nm absorbance changes (measured at 525 nm) in the wild type strain of Scenedesmus under aerobic and anaerobic conditions and in the absence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) are presented in Fig. 1. Results obtained with Scenedesmus are similar to those obtained by CHANCE

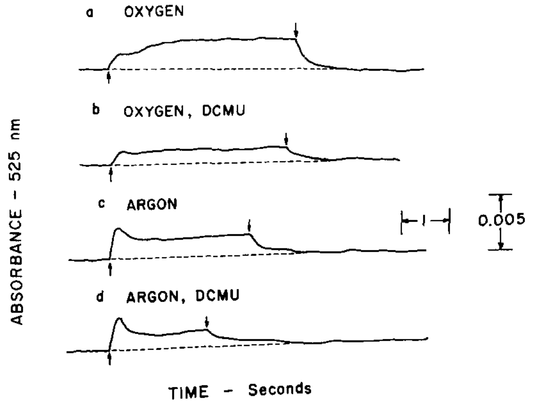


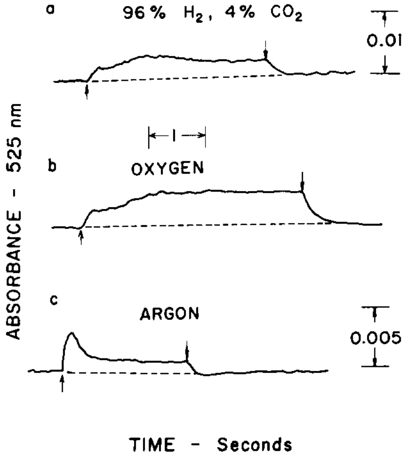
Fig. 1. Light-induced absorbance changes at 525 nm in suspensions of wild type Scenedesmus under 1 atm of argon or oxygen. 100 μl packed cell volume were washed and then suspended in 0.1 M $NaHCO_3$ and assayed in the presence or absence of 5 μM DCMU at 25°. Final volume was 6 ml.

AND STREHLER[6], WITT AND MORAW[16], and URBACH AND FORK[17] for Chlorella. Under oxygen two kinetically distinct absorbance increases occur, yielding a biphasic signal. Under argon the biphasic nature of the change disappears leaving only the first phase. DCMU (5 μM) completely inhibits the second phase of the absorbance change under aerobic conditions but does not significantly alter the first phase. There is no appreciable effect of DCMU on the uniphasic signal obtained under anaerobic conditions. The rise time of the first phase of the absorbance change is approx. 0.1 sec; that of the second phase approx. 1 sec.

Examination of the 520-nm change in suspensions of Chlorella and in an intact spinach leaf gave results identical to those presented in Fig. 1. The spinach leaf was treated with DCMU by repeated vacuum infiltration in a 30 μM solution of the inhibitor.

Four uncouplers and one inhibitor of photophosphorylation were examined for their effect on the 520-nm change. All five (50 μM NH_4Cl, 50 μM atebrin, 1 mM 2,4-dinitrophenol, 10 μM *m*-chlorocyanocarbonylphenylhydrazone (CCCP) and 2 μM antimycin A) had no effect on the absorbance change under either oxygen or argon.

By adapting Scenedesmus for photoreduction in an atmosphere of H_2–CO_2 (96:4, by vol.) it is possible to achieve the photoreduction of CO_2 without the participation of System II (ref. 18). Fig. 2 illustrates the 520-nm change which is observed in wild type Scenedesmus after adaptation for photoreduction. Sufficient DCMU (5 μM) was present to prevent the flow of electrons from System II to System I. The trace obtained under hydrogen, when compared to those obtained under oxygen and argon in non-adapted algae, is analogous to the biphasic signal obtained under oxygen. Although the second phase of the change is somewhat less distinct under hydrogen, it



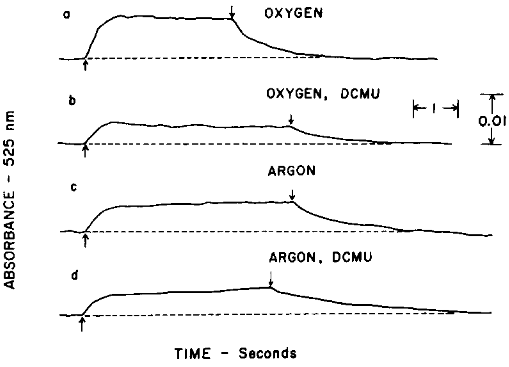


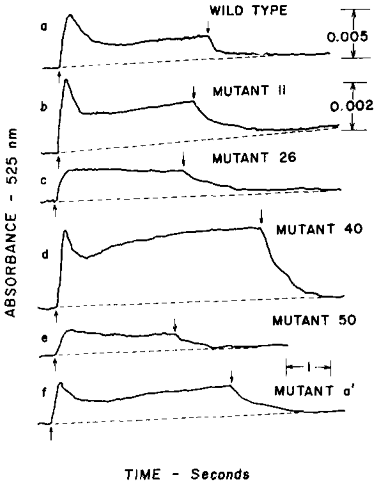
Fig. 2. Light-induced absorbance changes at 525 nm in wild type Scenedesmus at 25°. Upper trace: 100 μl packed cell volume were washed and then suspended in 5 ml 0.05 M potassium phosphate buffer (pH 6.7) with 2 moles $MgCl_2$/ml. DCMU was added to give 5 μM DCMU in 6 ml final volume. The cells were adapted overnight in H_2–CO_2 (96:4, by vol.) and assayed under the same atmosphere the following morning. Lower traces: 100 μl packed cell volume were washed, suspended in 0.1 M $NaHCO_3$ and assayed under 1 atm of oxygen or argon.

Fig. 3. Light-induced absorbance changes at 525 nm in suspensions of mutant 8 under 1 atm of oxygen or argon. 100 μl packed cell volume were washed, suspended in 0.1 M $NaHCO_3$ and assayed at 25° in the presence or absence of 5 M DCMU.

was observed consistently. An overshoot of the first phase, a characteristic occurrence under argon, was never observed in adapted algae under hydrogen.

The characteristics of the 520-nm change were examined in all six of the photosynthetic mutants. The results obtained with mutant 8 are presented in Fig. 3. A biphasic signal was never observed with this mutant, and only a moderate inhibition of the absorbance change was obtained with 5 μM DCMU under oxygen (as opposed to complete inhibition of the second phase in wild-type cells). No inhibition with DCMU was observed under argon. The appearance of the signal under oxygen was somewhat different from that under argon, but in both cases the rise time for the single phase of the absorbance increase was about 0.5 sec, intermediate between the rise times for the two phases in wild type cells under oxygen. A completely inhibited DCMU absorbance change was never obtained in whole cells of mutant 8, even in the presence of a Hill oxidant (*p*-benzoquinone).

The other five mutants (11, 26, 40, 50, and a′) also exhibited uniphasic changes under both oxygen and argon (Fig. 4). DCMU had no appreciable effect on the appearance of the signal in any of these mutants, although a moderate inhibition by DCMU was observed under oxygen. The absorbance change was kinetically the same under either gas phase, although it was enhanced slightly by anaerobic conditions. The rise time in all five mutants was approx. 0.1 sec, the same as that obtained for wild-type cells under argon. Two distinct types of signals were obtained with these mutants. The three System-II mutants (11, 40, and a′) all exhibited signals identical to that obtained for wild type cells under argon, consisting of a rapid spike followed by an



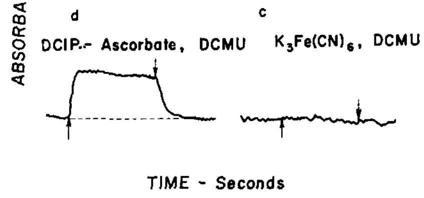


Fig. 4. Comparison of the light-induced absorbance changes at 525 nm in suspensions of wild type, System-II mutants (a′, 11 and 40) and electron-transport mutants (26 and 50) under 1 atm of argon. Note the difference in scale values for the mutant patterns. 100 μl packed cell volume employed for each phenotype.

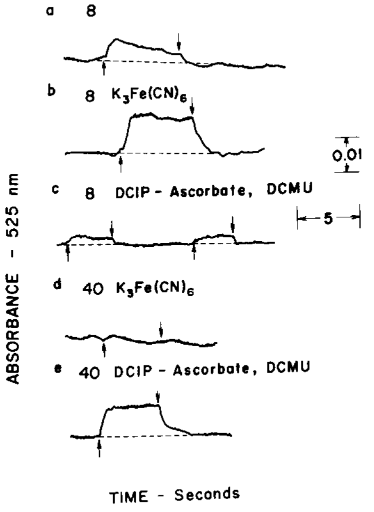
Fig. 5. Light-induced absorbance changes at 525 nm in chloroplasts from wild type Scenedesmus at room temperature in the presence of the following additions: trace a, no addition; trace b, 2 μmoles $K_3Fe(CN)_6$; trace c, as in trace b + 30 nmoles DCMU; trace d, 0.2 μmole DCIP, 20 μmoles ascorbate, 24 nmoles DCMU. All preparations contained 0.3 μmole potassium phosphate (pH 6.7), 12 μmoles $MgCl_2$, and 250 μg chlorophyll in a final volume after additions of 6 ml. (See text for details.)

absorbance decrease to a steady state level. The two electron-transport mutants (26 and 50) did not exhibit an initial spike; instead, the absorbance change reached a steady-state level directly.

Single-beam spectrophotometry (chloroplasts)

Two distinct kinds of 520-nm absorbance changes may be observed in chloroplast preparations from wild type Scenedesmus (Fig. 5). If no cofactors are added to wild type chloroplasts (trace a) a small absorbance increase is observed upon illumination. The addition of a Hill oxidant such as ferricyanide (trace b) stimulates the already apparent absorbance change. Other Hill oxidants, such as $NADP^+$, *p*-benzoquinone, and 2,6-dichlorophenolindophenol (DCIP) also stimulate the magnitude of the change. DCMU completely inhibits any absorbance change which is stimulated by the addition of oxidants (trace c). A second absorbance change which is not affected by DCMU is obtained upon the addition of a reductant (DCIP–ascorbate, trace d). Diaminodurol *plus* ascorbate also stimulated a DCMU-insensitive absorbance change. The DCIP–ascorbate induced absorbance change was partially inhibited by 20 μM CCCP (trace e). A biphasic signal was never observed with chloroplast preparations. Similar results were obtained with chloroplast preparations from spinach.

Fig. 6 illustrates the response of chloroplasts from the mutants to actinic illumination. Chloroplasts from mutant 8 exhibit a small absorbance increase (trace a) which is stimulated by the addition of an oxidant (trace b). The addition of DCMU completely inhibits the absorbance change stimulated by oxidants. Only a very small



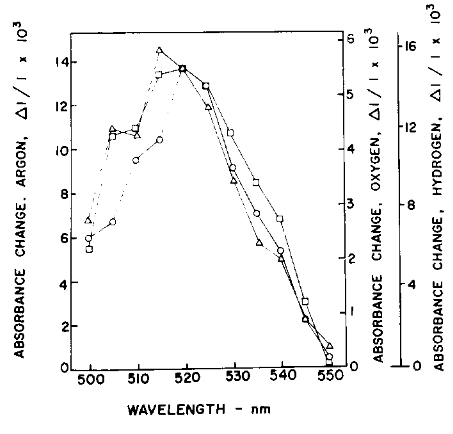


Fig. 6. Light-induced absorbance changes at 525 nm in chloroplasts from mutants 8 and 40, at room temperature, in the presence of the following additions; trace a, no addition; trace b, 2 μmoles $K_3Fe(CN)_6$; trace c, 0.2 μmole DCIP, 0.2 μmole diaminodurol, 20 μmoles ascorbate, 24 nmoles DCMU; trace d, as in trace b; trace e, as in trace c. All preparations contained 0.3 μmole potassium phosphate (pH 6.7), 12 μmoles $MgCl_2$ and 250 μg chlorophyll in a final volume after additions of 6 ml. (See text for details.)

Fig. 7. Difference spectra of the 520-nm light-induced absorbance change at 25° in wild type Scenedesmus under 1 atm of oxygen (△—△), argon (○—○) or hydrogen (□—□). 250 μl packed cell volume were washed and suspended in 6 ml of 0.05 M potassium phosphate buffer with 2 μmoles $MgCl_2$/ml.

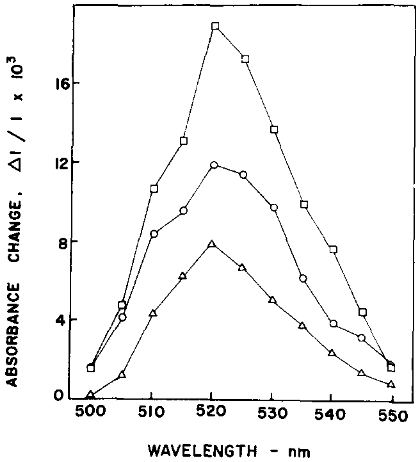
absorbance change is observed with chloroplasts from mutant 8 when reductants are added (trace c). Chloroplasts from the three System-II mutants (11, 40 and a′) and the two electron transport mutants (26 and 50) all exhibit identical responses to actinic illumination. Mutant 40 is used as an example in Fig. 6. No absorbance change is observed in the presence of oxidants (trace d) although an appreciable DCMU insensitive absorbance increase is noted in the presence of reductants (DCIP–ascorbate, trace e).

Difference spectra

The double-beam spectrophotometer was used to obtain the difference spectra. The actinic light was filtered through two red Corning glass filters (2-55, 2-63) and a 5-cm water bath. Its intensity was approx. $1.3 \cdot 10^5$ ergs·cm^{-2}·sec^{-1}.

Difference spectra, from 500 to 550 nm, were obtained for wild type Scenedesmus in the presence of oxygen and argon (Fig. 7). The difference peak for both spectra is approx. 520 nm. Under oxygen the absorbance change was consistently relatively larger at shorter wavelengths than that obtained under argon, although the difference was quite small.

The difference spectrum for the 520-nm absorbance change in wild type Scenedesmus adapted for photoreduction is also presented in Fig. 7 for comparison with those obtained under argon and oxygen. The difference spectrum under these conditions is similar to that obtained under oxygen. If the difference between the spectra under oxygen and argon is significant, then such a comparison further indicates that the absorbance change in adapted cells under hydrogen is analogous to that obtained in non-adapted cells under oxygen.



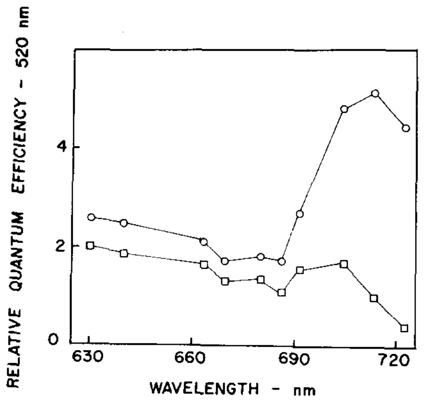


Fig. 8. Difference spectra for the 520-nm light-induced absorbance change at 25° in mutant strains 8 (△—△), 11 (○—○), and 26 (□—□) of Scenedesmus. The cells (250 μl packed cell volume) were washed, suspended in 6 ml of 0.05 M potassium phosphate (pH 6.7) with 2 μmoles $MgCl_2$/ml, and assayed under 1 atm of argon after settling to the bottom of the cuvette.

Fig. 9. Relative quantum efficiencies for the 520-nm light-induced absorbance change at 25° in wild type Scenedesmus from 630 to 722 nm under oxygen (□—□) or argon (○—○). Experimental conditions were the same as indicated in Fig. 7. The values plotted were obtained after 2 sec of actinic illumination. Shorter term measurements were not possible because of instrumental characteristics of the double-beam spectrophotometer (see METHODS).

Difference spectra for three of the photosynthetic mutants were also obtained. All three of the mutants, including a System-I mutant (8), a System-II mutant (11), and an electron-transport mutant (26), exhibit very similar difference spectra, peaking at 520 nm (Fig. 8). These spectra are similar to those obtained for wild type cells under argon and verify that the absorbance changes obtained in the mutants are true 520-nm absorbance changes and not scattering artifacts.

Relative quantum efficiency

Fig. 9 illustrates the relative quantum efficiencies obtained for the 520-nm absorbance change in wild type Scenedesmus under oxygen and argon. Thick suspensions of algal cells were used to insure complete absorption of the actinic light (greater than 99 %). Weak actinic illumination was used so that the absorbance change would not be saturated; intensities ranged from 3 to $5 \cdot 10^3$ ergs$\cdot$cm$^{-2}\cdot$sec^{-1}. Under argon the relative quantum efficiency remained constant throughout the region where both photosystems absorb (630–686 nm). In the region where only System-I pigments absorb (704–722 nm), the quantum efficiency is approximately doubled. Under oxygen the quantum efficiency again remained approximately constant throughout the region of absorption by both System I and II. However, at longer wavelengths the quantum efficiency dropped, indicating that light absorbed by System I alone is not as effective as light absorbed by both photosystems in producing a 520-nm absorbance increase under oxygen.

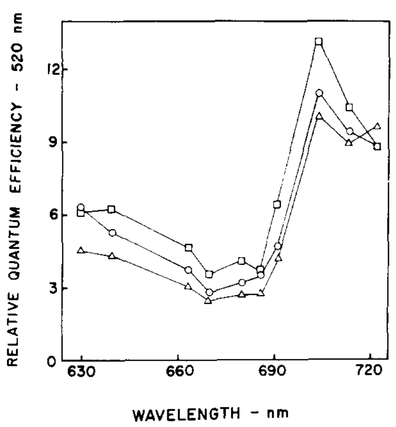


Fig. 10. Relative quantum efficiencies for the 520-nm light-induced absorbance change at 25° in mutant strains 8 (□—□), 11 (O—O), and 26 (△—△) of Scenedesmus.

The relative quantum efficiencies for the 520-nm absorbance change were assayed for three of the photosynthetic mutants (8, 11, and 26). All three of the mutants exhibited quantum efficiencies comparable to those obtained for wild type cells under argon (Fig. 10). The values in the region of 704–722 nm are approximately double those at shorter wavelengths for each mutant.

It must be stressed that the measurements reported in Figs. 9 and 10 are for a comparative or relative purpose rather than for an absolute. To obtain absolute values for the quantum efficiency would require the determination of absorption and scattering characteristics and a careful accessment of the linearity of signal magnitude as a function of intensity, at each of the wavelengths studied.

DISCUSSION AND CONCLUSIONS

The two phases of the 520-nm light-induced absorbance change which are observed under aerobic conditions may be separated by criteria other than their kinetic differences. The first (rapid) phase is relatively insensitive to DCMU, is stimulated by anaerobic conditions (except in hydrogen-adapted algae), and is sensitized primarily by System-I pigments. The second (slow) phase of the absorbance change, however, is completely inhibited by DCMU, is absent under anaerobic conditions (except in the presence of an electron donor), and is dependent upon System-II activity when water serves as the electron donor in photosynthesis (Figs. 1 and 9).

Previous investigators have postulated that a portion of the 520-nm absorbance change is a photoreduction mediated by System II (refs. 3 and 4). Their hypothesis is based largely on action spectra determinations and the influence of System-II inhibitors, notably DCMU. Presumably, this portion would be the second phase of the biphasic change observed in aerobic cells. Three observations, however, have led us to postulate that the entire 520-nm absorbance change, in intact cells, is mediated directly by System I. First, if System II were responsible for one phase of the absorbance change, then mutant 8 would be expected to exhibit an entirely DCMU-sensitive signal under oxygen, at least in the presence of *p*-benzoquinone, a Hill oxidant which stimulated oxygen evolution in this mutant. Such a signal was never obtained with mutant 8; the DCMU inhibition observed in this mutant was no greater than that found in the System-II and electron-transport mutants and was not comparable to that observed in wild type cells under oxygen where the entire second phase of the change was eliminated. Also, the 520-nm absorbance change observed in mutant 8 is not related to System-II activity, since it exhibits a preferential requirement for light absorption by System-I (*i.e.*, long wavelength absorbing) pigments (Fig. 10).

Second, the presence of both phases of the absorbance change in Scenedesmus adapted for photoreduction further suggests that System II is not required for either phase of the absorbance change and indicated that System I alone is capable of producing a biphasic absorbance increase at 520 nm. Finally URBACH AND FORK[17,19] have found that diaminodurol *plus* ascorbate will stimulate a biphasic absorbance change in Chlorella under anaerobic conditions and in the presence of DCMU, again indicating that System-I activity alone is sufficient for the appearance of both phases of the change. It would seem likely that diaminodurol and ascorbate are donating electrons to System I in intact Chlorella cells just as they are supposed to do in isolated chloroplasts.

If it is assumed that the second phase of the 520-nm absorbance change *in vivo* results from a substrate level photoreduction by System I, then the apparent dependence of this signal on System II (*cf.*, Fig. 9; GOVINDJEE AND GOVINDJEE[4], FORK, AMESZ AND ANDERSON[5]; RUBINSTEIN[3]) may be explained. None of the mutants could exhibit this phase of the change since they are incapable of transporting electrons from water to the reducing side of System I in substrate (as opposed to catalytic) amounts. Also, the effect of alternate electron donors (hydrogen and diaminodurol *plus* ascorbate)[17,19] in stimulating a biphasic change may be explained by suggesting that they substitute for water (and therefore System II) as electron donors to System I.

The first phase of the absorbance change may represent the same reaction as that producing the second phase although it is probable that it is an entirely different phenomenon. GOVINDJEE AND GOVINDJEE[4] and FORK, AMESZ AND ANDERSON[5] have already suggested that two chloroplast components are responsible for the complex nature of the 520-nm change. The appearance of an absorbance increase at 520 nm in algae lacking chlorophyll *b* supports this suggestion[21,22]. Also, the appearance of only the first phase of the absorbance change in mutant 8, even though both phases are apparently mediated by the same photosystem, would suggest that the two phases result from different reactions. The exact nature of the first phase of the change is not clear, but it is possible that it represents the photooxidation of a System-I pigment (perhaps a chlorophyll–carotenoid complex as suggested by MATHIS[8]) other than P700. The appearance of a DCMU-insensitive signal in mutant 8 probably results from a photooxidation mediated by the bulk chlorophyll of System I. The absence of P700 is manifested by the altered kinetics of the 520-nm signal in this mutant.

The difference spectra for the 520-nm change presented in Fig. 7 indicate that if two chloroplast components are responsible for the entire change[4,5], they have very similar difference spectra, at least in the region from 500 to 550 nm. The difference spectra presented by FORK, AMESZ AND ANDERSON[5] also suggest that the two components would have very similar spectra in this region. It is significant that the absorbance change in hydrogen-adapted algae is most similar to that obtained under oxygen since this adds support to the contention that the absorbance change is biphasic in algae performing photoreduction.

The absorbance changes observed in chloroplast preparations (Figs. 5 and 6) yield results different from those obtained in intact cells. The absorbance change observed in chloroplasts in the presence of Hill oxidants is apparently mediated by System II, although the comparable DCMU-sensitive absorbance change in whole cells (second phase) is apparently ultimately mediated by System I. Also, it is evident that the absorbance change in chloroplasts from mutant 8 is not comparable to that obtained in whole cells. The latter change is mediated by System-I pigments (Fig. 10) and is relatively DCMU insensitive while the former change is completely inhibited by DCMU and is apparently System-II mediated. Also, *p*-benzoquinone induces a typical DCMU-inhibited absorbance change in chloroplasts while it is totally inactive in stimulating an absorbance change in whole cells of this mutant. Further work needs to be done to explain these discrepancies, but it is possible that they are due, at least in part, to the disruption of the photosynthetic apparatus during the isolation procedure used to obtain the chloroplasts. One possible hypothesis to explain the different results obtained with intact cells, as opposed to chloroplasts obtained from the same cells, is that the second phase of the absorbance change represents the reduction of a pool of some chloroplast component which is available only to System I in intact chloroplasts but becomes available to System II when the chloroplasts are disrupted as they are during isolation. The data obtained from mutant 8 clearly indicate that results obtained with chloroplast preparations need not be indicative of activities *in vivo*. In this regard, it is interesting that LEVINE AND GORMAN[23] have recently reported the absence of a 520-nm change in specially prepared Chlamydomonas chloroplasts, apparently either in the presence of oxidants or reductants.

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