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Publication Date
1973-12-01
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December 5, 1973

Prepared for the U. S. Atomic Energy Commission under Contract W-7405-ENG-48

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ENERGY TRANSFER FROM PROTOCHLOROPHYLLIDE TO CHLOROPHYLLIDE DURING
PHOTOCONVERSION OF ETIOLATED BEAN LEAF HOMOGENATES

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SUMMARY

The photoconversion of protochlorophyllide to chlorophyllide in etiolated bean leaves or leaf homogenates exhibits complicated kinetics that is neither simple first-order nor second-order with respect to reactant. The source of this complexity appears to be the intermolecular transfer of electronic excitation energy from protochlorophyllide to chlorophyllide. At room temperature this occurs with increasing probability as the product chlorophyllide is formed and serves to decrease the quantum yield for protochlorophyllide photoconversion. Evidence for the excitation transfer is obtained during the course of photoconversion by comparing the chlorophyllide absorbance with the intensity of chlorophyllide fluorescence excited at wavelengths where both pigments absorb. Measurements of the polarization of chlorophyllide fluorescence indicate that efficient excitation transfer occurs at room temperature over pigment aggregates containing at least four molecules. The relative quantum efficiency of chlorophyllide-excited chlorophyllide fluorescence remains constant during photoconversion of homogenate or etioplast preparations. This result does not support the proposal of increasing exciton interaction between chlorophyllides during the course of photoconversion.

INTRODUCTION

The photochemical reduction of protochlorophyllide to chlorophyllide in etiolated angiosperm seedlings has the characteristics of a photoenzymatic reaction. It holds special interest because the photoconversion triggers a dramatic reorganization of the plant cell organelles
leading to the formation of photosynthetically active membranes characteristic of mature chloroplasts.

In an early study of the kinetics of the protochlorophyllide photoconversion in barley leaves, Smith and Benitez\(^3\) reported second-order behavior and suggested a requirement for collisional processes. Boardman\(^4\), using protochlorophyllide holochrome preparations, found the kinetics to be more consistent with a sum of two exponentials, which eliminated the need for intermolecular collisions, and he suggested that two environmentally distinct forms of protochlorophyllide are converted at different rates. Sironval \textit{et al.}\(^5\) found similar agreement with a sum of exponentials when the photoconversion was effected with 630 nm light, but found simple first-order behavior with 647 nm actinic light.

More recent studies have suggested that the kinetics of photoconversion is still more complex and that there is exact agreement with neither first- nor second-order formulations. Thorne and Boardman\(^6\) proposed that the complex kinetics arises from the decreasing efficiency of photoconversion as the reaction proceeds. They attributed this to the increasing efficiency of energy transfer from protochlorophyllide to the newly formed chlorophyllide--the probability of such energy transfer being proportional to the concentration of chlorophyllide. Nielsen and Kahn\(^7\) formulated a kinetic scheme which explicitly includes mechanisms for de-excitation of photoexcited protochlorophyllide. The mechanisms which they envisioned include first-order thermal de-excitation, self-fluorescence, and energy transfer to chlorophyllide (the rate for which is assumed to be linearly dependent upon the concentration of chlorophyllide). This formulation fits their data through 80-85% conversion. Nielsen and Kahn also reported
that the kinetics for photoconversion of holochrome subunits containing only a single pigment molecule is first-order.

Estimates of the size and nature of the molecular array over which energy transfer occurs differ widely, depending on the sample preparation used and the conditions under which measurements are made. Schopfer and Siegelman\(^8\), in isolating holochrome from barley, found two to four protochlorophyllide pigments per 600,000 molecular weight unit. Kahn et al.\(^9\) concluded on the basis of measurements of excitation transfer efficiencies at 77\(^\circ\)K that there are at least four pigment molecules per holochrome protein. Thorne\(^10\), however, working with intact etiolated leaves, found that excitation transfer at 77\(^\circ\)K appears to occur over as many as 20 molecules. Finally, Mathis and Sauer\(^11\) interpreted circular dichroism measurements on crude homogenate preparations from bean to demonstrate that protochlorophyllide molecules are associated as dimers.

The present study of etiolated and post-etiolated homogenate and etioplast preparations indicates that the kinetics is more complex than a sum of two exponentials. We suggest that pigment aggregates do indeed permit considerable excitation transfer, thus complicating the kinetics. Transfer of excitation from protochlorophyllide to chlorophyllide is demonstrated at room temperature, thus establishing a firm basis for kinetic formulations such as that of Nielsen and Kahn\(^7\). Furthermore, the fraction of protochlorophyllide excitation transferred to chlorophyllide increases as photoconversion of a sample proceeds. The transfer efficiency is very nearly linearly dependent on the concentration of chlorophyllide. Chlorophyllide fluorescence becomes progressively depolarized during the course of photoconversion, indicating a minimum
-5-

pigment group size of four chlorophyllide molecules. Finally, measurement of the relative quantum yield of chlorophyllide fluorescence during photoconversion indicates that the intrinsic fluorescence efficiency is constant—a finding that does not support the suggestion that pigments are closely associated as dimers.

MATERIALS AND METHODS

Kidney beans, disinfected with Arasan (duPont), were planted in vermiculite which had been briefly soaked in water. The seedlings were then grown in darkness at about 22°C for 11-13 days. Harvesting was done at room temperature under a green safelight; subsequent operations were carried out at 4°C in darkness or under the safelight. Each preparation was derived from leaves of the same age, and no variation among preparations that might be attributed to leaf age differences was detected.

Bean leaf homogenate was prepared by grinding about 20 g of leaves in 10 ml glycerol and 30 ml sucrose-tricine buffer consisting of 0.1 M tricine, pH 8.0, 0.4 M sucrose, for a total of 5 min in a Waring Blender. The 5-min homogenization period was divided into several shorter intervals to prevent overheating the sample. The brei was then filtered through four layers of cheesecloth and centrifuged for 30 min at 20,000 x g. The supernatant was dialyzed for 12 h against buffer diluted 10-fold, recentrifuged for 90 min at 20,000 x g, and concentrated by ultrafiltration against polyethylene glycol 6000. The resultant homogenate was then made to 1.2 M sucrose with a solution of 2.0 M sucrose in 0.1 M tricine, pH 8.0. The red absorption maximum of the homogenate was at 640 nm.
moving to 678 nm following photoconversion (see Fig. 1). No subsequent spectral shifts were observed at room temperature.

Etioplasts were prepared from etiolated bean leaves by grinding about 16 g of leaves in 50 ml buffer solution consisting of 0.1 M tricine, pH 7.5, 0.4 M sucrose, in a Waring Blendor for 45 sec. The homogenate was then filtered through eight layers of cheesecloth and centrifuged at 350 x g for 8 min. The pellet was washed twice with buffer, then resuspended in a minimum amount of 70% buffer solution/30% glycerol. The etioplast preparation showed an absorption maximum at 650 nm, shifting to 681 nm when photoconverted at -10°C. No dark shifts were seen following conversion at -10°C.

The fluorescence of chlorophyllide was usually measured in a Perkin-Elmer MPF-2A fluorometer. The emission monochromator was set at 685 nm (20 nm bandwidth) for the experiments in Figs. 2 and 3. For the rest of the experiments, the monochromator was set at 690 nm (40 nm bandwidth) and a 690 nm interference filter was placed in the emission beam. The wavelength and bandwidth of the excitation beam are given in the figure legends. The actinic light passed through several pieces of translucent tape so that the entire front surface of the cuvette containing the sample received the same illumination. The sample cuvette was covered with reflecting aluminum foil on the surfaces opposite the excitation and emission beams, and was cooled when necessary by passing cold N₂ gas through the sample block. In some of the experiments to be described, chlorophyllide fluorescence was also measured in a Cary 14 spectrophotometer fitted with a Model 1462 scattering accessory. In these instances, fluorescence was excited with 440 nm illumination which passed
through translucent tape and was measured through a Kodak #70 Wratten filter which blocked the actinic beam as well as all emission below about 660 nm. For fluorescence measurements, the Cary 14 was operated in the reference mode with a linear (% T) slidewire. The jacketed sample cuvette was cooled with cold N₂ gas.

Absorption measurements were made on the Cary 14 with an expanded scale (0.0 - 0.2 absorbance) slidewire. The standard sample compartment was used except for the absorption spectra of etioplast suspensions, where the Model 1462 scattered transmission accessory was used.

Fluorescence polarization measurements were made using the Perkin-Elmer instrument as described above, except that aluminum foil covered only the side of the cuvette opposite the excitation beam. Fluorescence polarization measurements on solutions of fluorescein demonstrated that the presence of the foil had no effect on the results. A Polaroid polarizer was oriented in the excitation beam normal to the plane defined by the excitation and emission beams. A second polarizer was used to analyze the emission into parallel and perpendicular components. The excitation monochromator was set at 670 nm (4 nm bandwidth). Corrections were applied to the polarization results as described by Houssier and Sauer¹².

RESULTS

Kinetics. Etiolated leaf homogenates in 1.2 M sucrose were photoconverted in the fluorometer at room temperature with illumination at various wavelengths. As the photoconversion proceeded, the fluorescence of chlorophyllide excited by the actinic beam was measured continuously. At regular
intervals the sample was removed to a spectrophotometer and the absorption spectrum recorded. At the end of the experiment, complete conversion was effected by exposing the sample to intense illumination at 640 nm for about 5 min. A final fluorescence value and a final absorption spectrum were then recorded. Thus, both absorption changes and chlorophyllide fluorescence at 685 nm were obtained as a function of time of illumination. The results of five such experiments are shown in Fig. 2, each symbol type representing a single experiment in which absorption at 678 nm and chlorophyllide fluorescence at 685 nm (excited at the indicated wavelength) were measured. The half times of the experiments varied by a factor of two owing to variations in light intensity at the sample and differences in effectiveness among the actinic wavelengths in promoting photoconversion. The time scales, therefore, were linearly adjusted so that the curves representing absorption change coincided (see figure legend).

The high concentration of sucrose in the sample insured the stability of the 678 nm form of chlorophyllide. However, a small fluorescence decrease attributable to dark processes was observed. This fluorescence decrease probably has its origin in the same processes that cause the decrease in chlorophyllide fluorescence yield observed by Thorne following photoconversion in intact leaves. These processes are apparently substantially slowed in sucrose homogenate preparations. To illustrate this phenomenon, and to indicate how corrections were made, Fig. 3 depicts a typical fluorescence trace vs. time of illumination. The discontinuities occur at those times when the sample was removed from the fluorometer to allow recording of the absorption spectrum. These intervals were long
(about 5 min) compared to the times of illumination. Consequently, we concluded that, during the course of the brief illumination periods, no significant decrease occurred in the fluorescence of previously formed chlorophyllide. Thus, the corrected fluorescence at any time was taken as the measured fluorescence plus the sum of decreases observed during the intervals between illumination periods. The dotted line in Fig. 3 illustrates the level of corrected fluorescence. (Although this method of correction cannot be defended rigorously, we believe that the conclusions based on these measurements would not be materially altered even in the absence of any corrections.)

In general, experimental curves such as those represented in Fig. 2 (with the exception of the curve representing chlorophyllide fluorescence excited at 640 nm) could be computer fit with high accuracy with a sum of two exponentials. However, there are several indications that such fits are fortuitous. First, for a reaction representable by a sum of two exponentials, any measured property of chlorophyllide, such as absorbance or fluorescence intensity, will be resolvable in terms of only two rate constants. Consequently, if two properties of chlorophyllide are measured during the photoconversion of a single sample, the time courses of both properties should be resolvable in terms of the same two rate constants, though perhaps with different preexponential factors. However, such was never the case in experiments such as those illustrated in Fig. 2. Second, curves were artificially generated by sums of three or four exponentials in which rate constants differed by factors as much as five. The computer program used could usually fit such curves with only two exponentials and with remarkable precision.
Third, the curve representing chlorophyllide fluorescence excited at 640 nm could not be fit by the sum or difference of any two exponentials.

Fig. 2 also illustrates another striking phenomenon. The curves representing chlorophyllide fluorescence excited at 587 nm, 628 nm, 640 nm, and 650 nm all rise more rapidly than does the curve representing chlorophyllide absorbance (which may be taken as a measure of chlorophyllide concentration\(^{11}\)). It is particularly notable that this disparity is greatest at 640 nm, the absorption maximum of protochlorophyllide.

**Chlorophyllide fluorescence yield.** To confirm that the observations described above were not affected by a real change in the intrinsic quantum yield of chlorophyllide fluorescence during photoconversion, an experiment was conducted in which chlorophyllide fluorescence (at 690 nm) excited at 670 nm was measured at room temperature in addition to absorbance at 678 nm and fluorescence excited at 640 nm. Excitation of the homogenate preparation at 670 nm caused no photoconversion, and chlorophyllide may be considered to be the only absorbing pigment. The results of two such experiments are plotted in Fig. 4. From the coincidence of the curves representing chlorophyllide absorption at 678 nm and intrinsic chlorophyllide fluorescence \((E_{670})\), we conclude that the intrinsic quantum yield of chlorophyllide fluorescence is constant during the course of photoconversion in the sucrose homogenate. To extend this conclusion to etioplast preparations, a somewhat more complex set of experiments was necessary. In order to prevent subsequent dark shifts in the chlorophyllide absorption, it was necessary to cool the etioplasts to \(-10^\circ\text{C}\), thus it became inconvenient to move the preparation from one instrument to another during the course of
a single experiment. Therefore, fluorescence at 690 nm excited at 440 nm and at 670 nm were measured during photoconversion in the fluorometer, and fluorescence excited at 440 nm and absorbance were similarly measured on a second sample under similar conditions in the Cary spectrophotometer. The results of four such experiments on the fluorometer and two on the Cary 14 are summarized in Fig. 5. The six curves representing chlorophyllide fluorescence excited at 440 nm (one from each of the six experiments) have been made to coincide by multiplying the time scales of the six experiments by appropriate factors. This allows the direct comparison of the time courses of intrinsic chlorophyllide fluorescence (excited at 670 nm) and chlorophyllide absorbance at 681 nm. As with the homogenate (Fig. 4), this comparison reveals that the quantum yield of chlorophyllide fluorescence remains substantially constant during the photoconversion.

Polarization of fluorescence. The polarization of chlorophyllide fluorescence excited at 670 nm was measured at room temperature during the course of photoconversion of a leaf homogenate preparation. The fluorescence polarization may be expressed as

\[ P = \frac{I_\parallel - I_\perp}{I_\parallel + I_\perp} \]

where \( I_\parallel \) and \( I_\perp \) are the measured intensities of fluorescence polarized parallel and perpendicular to the polarized actinic beam. The results for two such experiments, which include the corrections for monochromator anomalies, are plotted in Fig. 6. The error bars represent the approximate experimental uncertainty in each measurement. In addition, the results indicate that there may have been a systematic difference between the
two sample preparations used. However, a significant trend in the fluorescence polarization is obvious. The polarization decreases from a value of $0.38 \pm 0.08$ at 6% conversion to $0.17 \pm 0.05$ at 50% and $0.11 \pm 0.03$ at 100% conversion.

DISCUSSION

Energy transfer from protochlorophyllide to chlorophyllide at 77°K has been demonstrated by several investigators $^9, ^{10}$ and has been proposed to occur at room temperature in recent kinetic schemes $^6, ^7$. The occurrence of such excitation transfer at room temperature is the most straightforward explanation for the observations illustrated in Fig. 2. We propose that the relatively high levels of chlorophyllide fluorescence excited at 587 nm, 628 nm, 640 nm, and 650 nm during the early stages of photoconversion arise from the fact that, while some of the actinic illumination absorbed by protochlorophyllide at these wavelengths promotes photoconversion, some of the energy is transferred to the newly formed chlorophyllide. Thus the chlorophyllide fluorescence excited at wavelengths shorter than 660 nm is dependent not only upon the concentration of chlorophyllide but also upon the concentration of photoactive protochlorophyllide. When chlorophyllide emission is excited by direct absorption of actinic illumination, and at the low sample concentrations used, chlorophyllide fluorescence is proportional to the concentration of the pigment and shows the same time course as absorption (see Fig. 4). Therefore, the disparity between the curve representing chlorophyllide absorption and those representing fluorescence in Fig. 2 arises from the contribution of energy absorbed by active protochlorophyllide and transferred to the chlorophyllide. This is confirmed by the observation
that this disparity is greatest at 640 nm, the absorption maximum of the protochlorophyllide in the homogenate preparation, and is smallest at 587 nm and 628 nm, where the excitation spectrum of chlorophyllide fluorescence in the completely converted homogenate has maxima. If it is assumed that only active protochlorophyllide and chlorophyllide absorb at 650 nm, the above conclusions can be used to calculate energy transfer efficiencies from the curve representing chlorophyllide fluorescence excited at 650 nm and that representing chlorophyllide absorbance (Fig. 2). At any point during the photoconversion, the extent, $E_t$, of energy transfer to chlorophyllide, expressed as a percentage of the energy absorbed by active protochlorophyllide, can be calculated from the relation

$$E_t = \frac{E_{650} - E_{670}}{E_{670}} \cdot \frac{\epsilon_{\text{Chlide}}}{\epsilon_{\text{PChlide}}} \cdot \frac{[\text{Chlide}]}{[\text{PChlide}]} \cdot 100$$

where $E_{650}$ and $E_{670}$ are the intensities of chlorophyllide fluorescence excited at 650 nm and 670 nm, $\epsilon_{\text{Chlide}}$ and $\epsilon_{\text{PChlide}}$ are extinction coefficients for chlorophyllide and active protochlorophyllide at 650 nm, and the brackets denote concentrations. The straightforward calculation of $E_t$, however, is beset by two uncertainties. First, since the bandwidth of the actinic light was substantial (10 nm), the relative values of the extinction coefficients at 650 nm do not rigorously represent the relative absorption efficiencies of the two pigments. Second, the inactive protochlorophyllide, with an absorption maximum at about 630 nm, makes a small contribution to the absorption in the region of 650 nm. With cognizance of these uncertainties, the efficiency of energy transfer, $E_t$, has been estimated from the above equation using $\epsilon_{\text{Chlide}}/\epsilon_{\text{PChlide}} = 0.58 \pm 0.08$. These calculations are summarized in Table I.
Thorne and Boardman\textsuperscript{6}, and Nielsen and Kahn\textsuperscript{7} have suggested that the probability of energy transfer from protochlorophyllide to chlorophyllide is linearly dependent upon the concentration of chlorophyllide. This assertion is verified by the values of $E_t$ in Table I. Using the value of $E_t$ at 10\% conversion, transfer efficiencies at further conversion were calculated assuming that the probability of such transfer increases linearly with the concentration of chlorophyllide. These theoretically derived values for transfer efficiency, $E_{t,\text{theory}}$, are also shown in Table I. The agreement between the latter values and the experimentally observed values for $E_t$ provides strong empirical support for the kinetic formulation of Nielsen and Kahn\textsuperscript{7}.

In the absence of rotational diffusion, the polarization of emission from isolated, randomly oriented absorption dipoles is 0.50. As expected, the polarization of chlorophyllide fluorescence in homogenates approaches this value at low conversion. With increasing conversion, the progressive decrease in polarization values suggests that absorbed energy is transferred among pigments before emission. Since the extent of fluorescence depolarization is dependent upon the relative orientation of pigment dipoles and upon the efficiency of energy transfer among the pigments, the number of molecules among which excitation transfer occurs cannot be deduced from the fluorescence polarization results. However, the results in Fig. 6 require that aggregates permitting efficient intermolecular energy transfer contain at least four pigment molecules. Approximately 75\% of the polarization decrease observed during the course of photoconversion occurs before 50\% conversion is reached. This result cannot be explained by a model involving three or fewer pigment molecules, even if dipole
orientations and excitation transfer efficiencies are chosen to maximize depolarization. Indeed, the results are most adequately fit if average group sizes are assumed to be five or larger.

The fluorescence polarization of chlorophyllide-protein holochrome in 2 M sucrose has been measured by Schultz and Sauer\textsuperscript{13}. They concluded that the observed depolarization (relative to monomeric chlorophyllide in viscous solvents) was due to energy transfer between pigments on the holochrome protein. The present results at 100\% conversion agree qualitatively with those of Schultz and Sauer, though the depolarization observed in the present work is more pronounced. One possible reason for this disparity is that the sucrose homogenate used in the present work contains larger protein aggregates than the purified holochrome preparation of Schultz and Sauer. Thus, more extensive energy transfer may be possible. Measurements of chlorophyllide fluorescence polarization in which no substantial depolarization was observed\textsuperscript{14,15} were probably performed on preparations in which pigment disaggregation had occurred following photoconversion (see Ref. 13).

Finally, the constancy of the intrinsic quantum yield of fluorescence of chlorophyllide during photoconversion does not confirm a model in which closely coupled dimers of chlorophyllide exist\textsuperscript{11}. Such dimers would be expected to display a fluorescence efficiency different from that of the monomers (i.e., mixed chlorophyllide-protochlorophyllide dimers) present at partial conversion. Changes in the shape or position of the absorption band during photoconversion are also absent\textsuperscript{11}. In the absence of closely coupled dimers, however, the CD spectra of Mathis and Sauer cannot be readily explained. This apparent discrepancy remains unresolved at present.
CONCLUSION

Our findings provide conclusive support for some of the more recent proposals concerning photoconversion kinetics, pigment aggregation, and energy transfer. The current picture may be outlined as follows:

1) Under conditions that inhibit post-conversion "dark shifts," the protochlorophyllide and chlorophyllide pigments appear to be substantially associated during and following photoconversion.

2) Energy transfer from protochlorophyllide to chlorophyllide competes effectively with the photoconversion process at room temperature and appears to be the primary complicating factor in the kinetics of the photoconversion.

3) The nature of the pigment association does not manifest the usual characteristics of excitonic interactions, although it does produce a non-conservative, double CD spectrum. The excitation transfer leading to chlorophyllide emission and its resulting depolarization is probably of the longer range type described by Förster 16.

ACKNOWLEDGMENTS

This work was supported, in part, by the U. S. Atomic Energy Commission, and, in part, by a grant from the National Science Foundation (GB-24317).

REFERENCES

3 Smith, J.H.C. and Benitez, A. (1964) Plant Physiol. 29, 135-143
TABLE I

THE EFFICIENCY, \( E_t \), OF ENERGY TRANSFER FROM PROTOCHLOROPHYLLIDE TO CHLOROPHYLLIDE AT ROOM TEMPERATURE, EXPRESSED AS PERCENTAGE OF THE ENERGY ABSORBED BY ACTIVE PROTOCHLOROPHYLLIDE

\( E_{650} \) and \( E_{670} \) are the relative values of chlorophyllide emission at 685 nm excited at 650 nm and 670 nm, respectively, as abstracted from Fig. 2 (\( E_{670} \) is assumed to be proportional to \( A_{678} \)—see text and Fig. 4); \([\text{Chlide}]/[\text{PChlide}]\) is the ratio of the concentrations of chlorophyllide and active protochlorophyllide. \( E_t \) is then calculated for \( E_{\text{Chlide}}/E_{\text{PChlide}} = 0.58 \pm 0.08 \) according to Eq. (1). \( E_{\text{theory}} \) are the calculated values for the efficiency of energy transfer based on \( E_t \) at 10% conversion and the assumption that the probability of transfer increases linearly with the concentration of chlorophyllide.

<table>
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<th>% Conversion</th>
<th>( E_{650} - E_{670} )</th>
<th>([\text{Chlide}]/[\text{PChlide}])</th>
<th>( E_t )</th>
<th>( E_{\text{theory}} )</th>
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<td>0.11</td>
<td>13±2</td>
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<td>27±5</td>
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<td>3.00</td>
<td>56±8</td>
<td>53</td>
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FIGURE CAPTIONS

Fig. 1. The absorption spectrum of an etiolated bean leaf homogenate in sucrose, before and after complete photoconversion.

Fig. 2. Absorbance and fluorescence of chlorophyllide in leaf homogenates as a function of time of illumination at room temperature. Upper four curves from top to bottom: chlorophyllide fluorescence at 685 nm excited at 640 nm (8 nm bandwidth), 650 nm (10 nm), 628 nm (8 nm), and 587 nm (14 nm) respectively. Lower curve: absorbance change at 678 nm. Each symbol type represents a single experiment in which absorbance change and fluorescence were measured. The half time for each experiment, as measured by $A_{678}$, has been adjusted to the same value by multiplying each time scale by the appropriate factor. The true half times of the experiments were between 70 and 120 s.

Fig. 3. A typical trace of chlorophyllide fluorescence at 685 nm excited at 640 nm vs. time of illumination at room temperature. The discontinuities represent the points at which absorption spectra were taken. The decrease in fluorescence between intervals of illumination was assumed to result from a dark process which slightly reduced the fluorescence yield of chlorophyllide. The broken line is the fluorescence intensity corrected for this dark decay (see text). At A, complete photoconversion was effected.

Fig. 4. Absorbance and fluorescence of chlorophyllide in leaf homogenates as a function of time of illumination at room temperature. The symbols represent the averages of two experiments. The range of experimental values is shown by the error bars. $\Delta$, chlorophyllide fluorescence at
690 nm excited at 670 nm (3.5 nm bandwidth); o, absorbance change at 678 nm; □, chlorophyllide fluorescence at 690 nm excited at 640 nm (10 nm).

Fig. 5. Fluorescence and absorbance of chlorophyllide as a function of time of illumination of etioplast preparations at -10°C. Each symbol type represents a separate measurement: the solid symbols represent measurements on the Cary 14 of absorbance at 681 nm (lower curve) and chlorophyllide fluorescence excited at 440 nm (10 nm bandwidth) (upper curve); the open symbols represent measurements on the fluorometer of chlorophyllide fluorescence at 690 nm excited at 670 nm (3.5 nm bandwidth) (lower curve) and fluorescence at 690 nm excited at 440 nm (5 nm bandwidth) (upper curve). The time scale for each experiment has been multiplied by an appropriate factor so that the six curves representing fluorescence excited at 440 nm coincide.

Fig. 6. Polarization of chlorophyllide fluorescence at 690 nm excited at 670 nm (4 nm bandwidth) as a function of extent of photoconversion of homogenate preparation. Each symbol type represents a separate experiment at room temperature. The error bars represent the estimated uncertainty in each measurement.
BEFORE ILLUMINATION
AFTER COMPLETE CONVERSION

Fig. 1.
Fig. 2.
Fig. 3.
Fig. 4.
Fig. 5.
Fig. 6.
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