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DELAYED LIGHT STUDIES ON PHOTOSYNTHETIC ENERGY CONVERSION

I. IDENTIFICATION OF THE OXYGEN-EVOLVING PHOTOREACTION AS THE DELAYED LIGHT EMITTER IN MUTANTS OF *SCENEDESMUS OBLIQUUS*

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SUMMARY

1. The relative intensity of delayed singlet emission from chlorophyll was measured from 10^{-3} sec to 10^{2} sec after cessation of illumination in the wild-type and two non-photosynthetic mutants of a green alga, *Scenedesmus obliquus*.

2. Mutant 11, in which the oxygen-evolving, short-wavelength photoreaction (photoreaction II) was blocked, emitted very low intensities of delayed light compared with the wild-type. When the emission was averaged from 0.75 to 4.2 msec from the center of a flash of exciting light, the wild-type emitted 250 times the intensity emitted by mutant 11.

3. Mutant 8, in which the pyridine nucleotide-reducing, long-wavelength photoreaction (photoreaction I) was blocked, emitted delayed light with different decay kinetics from those of the wild-type. In the millisecond time range, mutant 8 emitted higher intensities of delayed light, and the emission decayed very slowly. In the second time range, mutant 8 emitted lower intensities than the wild-type, and the decay was rapid.

4. On the basis of the two-photoreaction hypothesis, and of the earlier evidence from photosynthetic bacteria which indicates that functional reaction centers are required in order for delayed light to be emitted, the weak emission from mutant 11 implies that nearly all delayed light from normal plants is due to energy storage by photoreaction II.

5. The altered decay kinetics of mutant 8 (higher intensity, slower decay) in the msec time range are shown to be consistent with the HILL AND BENDALL hypothesis of two photoreactions linked by an electron-transport chain. Lack of function of photoreaction I would result in a completely reduced electron-transport chain which could not accept reducing equivalents from photoreaction II.

6. The altered decay kinetics of mutant 8 in the second time range are shown to be inconsistent with any modification of the HILL AND BENDALL hypothesis which does not provide a pathway for reducing equivalents to migrate from photoreaction I to photoreaction II. A comparison of this long-term delayed light in the wild-type

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and mutant 8 implies the presence of a slow charge migration (1–50 sec) in the wild-type from photoreaction I to photoreaction II, which in turn results in long-term light emission by photoreaction II.

7. Two modifications of the HILL AND BENDALL hypothesis, both of which provide for appropriate charge migration, are discussed. The present data may be accounted for either by the addition of a second electron-transport chain or by a single inhomogeneous pigment system which harvests energy from two spatially separated reaction centers. The latter hypothesis also accounts for a number of previously published observations.

INTRODUCTION

The delayed singlet emission from chlorophyll of living plants allows the study of electron transitions over a wide time range within the functioning photosynthetic apparatus[1–6]. The present series of investigations is an attempt to understand the details of this delayed light emission, and thereby to clarify certain aspects of the energy conversion processes of photosynthesis. The observations are best understood in terms of energy storage by the photosynthetic apparatus. The stored energy is used to produce chemical potential for photosynthetic electron-transport enzymes. The delayed light is due to a low-yield reexcitation of chlorophyll by the stored energy. The intensity of delayed light emission at any time after a flash of exciting light is thus a measure of the amount of energy which is stored at that particular time. In order to understand the overall shape of the delayed light dark-decay, it seems necessary to invoke solid-state mechanisms for the energy storage and for the re-excitation processes[7]. These solid-state mechanisms must be coupled to the enzymatic reactions of electron transport.

It is now clear that more than one photoreaction* modulates the delayed light emission of living plants, since differential excitation of chlorophyll *a*, or of accessory pigments, affects the intensity and decay characteristics of delayed light emission[5,7–9]. This observation is in agreement with many other lines of evidence which indicate the presence of at least two different photoreactions in the energy conversion steps of photosynthesis[10–23]. If we accept the two-photoreaction hypothesis of HILL AND BENDALL[17,18], we may ask: What is the relative intensity of delayed light emission from each photoreaction? The present study answers this question by the use of Scenedesmus mutants in which each photoreaction is selectively blocked, the other photoreaction being left intact.

We found that nearly all the emission is due to energy storage by the short-wavelength photoreaction (photoreaction II), as defined by DUYSENS, AMESZ AND KAMP[23]. Several modifications of the HILL AND BENDALL hypothesis are discussed

* We use the term photoreaction to denote a complete mechanism in which absorption of light results in production of chemical free energy. The term pigment system denotes groups of co-operating pigment molecules in which electronic excitation energy may migrate between all the molecules before being dissipated as fluorescence or otherwise lost (about 10^{-9} sec in plants). The term excitation migration denotes this type of energy transfer, in order to separate it from the slower phenomenon of charge migration. The term reaction center denotes a specific site at a chlorophyll–enzyme interface where chemical free energy is delivered to enzyme systems as oxidizing and reducing equivalents, which means that a reaction center is a site where electrons and holes move between the pigment system and the enzyme systems of a photoreaction.

in light of the altered decay kinetics of the mutant blocked in the long-wavelength photoreaction (photoreaction I). The altered kinetics imply a slow (1–50 sec) charge migration from photoreaction I to photoreaction II which does not involve the HILL AND BENDALL electron-transport chain.

EXPERIMENTAL

The biological material

We studied the delayed light emission of the wild-type and two X-ray-induced mutants, 8 and 11, of *Scenedesmus obliquus*, Gaffron's strain D_3. These strains of algae have been characterized by BISHOP and collaborators[24–27] and the pertinent data about each strain are summarized in Table I. BISHOP and collaborators conclude that in mutant 8 the pyridine nucleotide-reducing, photoreaction I is non-functional, whereas the oxygen-evolving, photoreaction II is intact. They also conclude that in mutant 11 photoreaction I is intact, whereas photoreaction II is non-functional.

TABLE I

CHARACTERISTICS OF THREE STRAINS OF *Scenedesmus obliquus* D_3*

	Wild-type	*Mutant 8*	*Mutant 11*
Autotrophic growth (photosynthesis)	+	o	o
Heterotrophic growth (dark + glucose)	+	+	+
Pigmentation	Normal	Normal	Normal
Respiration	Normal	Normal	Normal
Presence of hydrogenase	+	+	+
Photoreduction of CO_2 with H_2	+	o	+
Quinone Hill reaction	+	+	o
Presence of P-700	+	o	+
Presence of narrow, rapid electron spin resonance signal	+	o	+
Presence of broad, slow electron spin resonance signal	+	+	o

* After BISHOP and collaborators[24–27].

The three strains of algae were grown heterotrophically in the dark at 21° for 3 days on BISHOP's Scenedesmus medium. The cultures were shaken at a frequency of 50 cycles per min. Before being placed in the delayed light apparatus, the cultures were concentrated to a standard cell concentration of 3 mm^3 of cells per ml of suspension. The cell suspension was then placed in the delayed light apparatus, and the measurement taken at 20°. When delayed light decay curves were measured, the cells were first given a 2-min illumination period at full intensity (see below) in order to reduce the effects of dark → light transients.

Experimental apparatus

In order to cover the time range from 10^{-3} to 10^2 sec, two instruments were used to measure the delayed light emitted by the three strains of *S. obliquus*. A modification of the Becquerel phosphoroscope allowed measurement of delayed light from 1 to 80 msec after the middle of a repeating flash of light. A single-exposure

shutter allowed measurement of delayed light from 0.7 to 100 sec after continuous illumination.

The phosphoroscope. Fig. 1 is a schematic diagram of our modification of the Becquerel phosphoroscope. The instrument consisted of a pair of spinning discs mounted on a common shaft, between which the cell suspension was held stationary in a 2-ml cellulose nitrate test tube. In order to provide adequate light-baffling, the discs were mounted so that they spun in narrow slots within an aluminum housing.

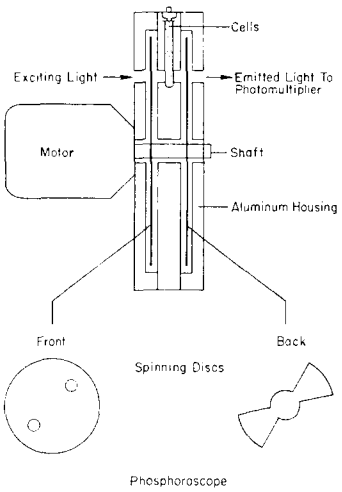

Fig. 1. Schematic diagram of the phosphoroscope. The cell suspension received repeating flashes of exciting light through the holes in the front disc. During each bright flash the photomultiplier was protected by the blades of the back disc, but between flashes the photomultiplier viewed the emission from the cells.

The discs and the slots were painted with flat black paint (3 M Co., Velvet Coating No. 9564). The cells were illuminated through two oppositely spaced holes in the front disc, so that the cells received two evenly spaced flashes of exciting light per revolution of the discs. The cells were viewed by a photomultiplier placed behind the back disc. This back disc was cut into the shape of a paddle wheel, the blades of which were aligned to coincide with the illuminating holes of the front disc. The back disc thus allowed the cells to be viewed by the photomultiplier for a period between each exciting flash, but for the duration of each flash the blades of this disc protected the light-measuring equipment from the bright exciting light.

Various portions of the delayed light decay curve between 1 and 80 msec were obtained by running the motor at different speeds. Our fastest measurement with this instrument (1 msec) was taken when the cells received 250 flashes of exciting light per sec, and our slowest measurement (80 msec) was taken when the cells received 10 flashes per sec. The cells received the same total energy of exciting light per sec at the various speeds because the duration of each individual flash of exciting light was longer at the slower speeds. Thus, when the plants received two different numbers of flashes per sec, measurement of the delayed light emission at one time after the centers of the exciting flashes resulted in a shorter period of complete darkness at the slower speed. This causes, at any particular time after the center of the flashes, an

increased delayed light emission at the slower speed so that the decay curves taken at various speeds do not overlap (*cf.* Fig. 2).

In order to reduce excitation of pigments other than chlorophyll, the exciting light consisted of the wavelength region 6500–12000 Å. The filament of a 1500-W tungsten bulb was focused on the cells to give an image of about 1 cm^2, and the bulb's incandescent emission was filtered through 7 cm of water and Corning glass color filter CS 2-64. This light saturated the delayed light emission at all dark decay times which we measured.

The photomultiplier was an RCA 7102, cooled with liquid N_2 and operated between 700 and 1400 V. The signal from the photomultiplier could be fed into any one of three different detection systems, according to the purposes of the particular experiment. The decay of emission could be observed directly on a monitor oscilloscope (Tektronix 545 A, Type D high-gain differential plug-in unit). Alternately, a smoothed decay curve could be obtained by averaging several thousand individual decay curves on a digital memory oscilloscope (Northern Scientific Model 513). Our most sensitive measurement was made without the oscilloscope by feeding the photomultiplier signal directly into an electrical filter (10 sec time constant) and subsequently monitoring the averaged signal with a vibrating-reed electrometer coupled

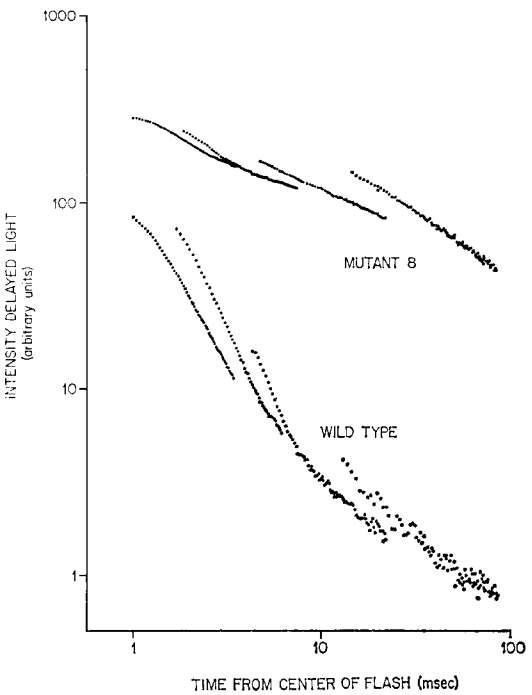

Fig. 2. Dark-decay curves of delayed light emission in the msec time range. Data were taken with the phosphoroscope–digital memory oscilloscope apparatus. The points are the signals stored in individual channels of the memory. Decay curves are given for four different phosphoroscope speeds: 250 flashes/sec (1–3 msec), 100 flashes/sec (1.5–5.5 msec), 30 flashes/sec (4.5–20 msec), 10 flashes/sec (14–80 msec). The curves taken at different speeds do not overlap because of changes in duration of the exciting light (see text). Mutant 8 emitted from three times (1 msec) to 50 times (80 msec) more delayed light than the wild-type. Mutant 11 did not emit detectable intensities of delayed light.

to a Brown recorder. With this detection system it was possible to measure extremely low light intensities, but the resulting data reflect only an average emission during the time that the back disc was open. These data thus give little information about the decay kinetics of the emission.

The single-exposure shutter. The shutter used to measure delayed light from 0.7 to 100 sec after illumination has been described previously[7]. The apparatus consisted of an entrance shutter which, upon being closed, simultaneously extinguished an exciting light and activated a trigger mechanism so that an exit shutter fell open under gravity. A photomultiplier (RCA 7102 operated as described above) could then view the light emission of a cell suspension which was held between the two shutters. The photomultiplier signal was amplified and then recorded on a chart giving relative delayed light intensity *versus* time after complete closure of the entrance shutter. The exciting light was similar to that described above.

RESULTS

Fig. 2 shows the delayed light emission from 1 to 80 msec from *S. obliquus* D_3 wild-type and mutant 8, as measured by the phosphoroscope–digital memory oscilloscope apparatus. The emission from mutant 11, in which photoreaction II was blocked, was not detectable with this equipment. Emission from the wild-type was

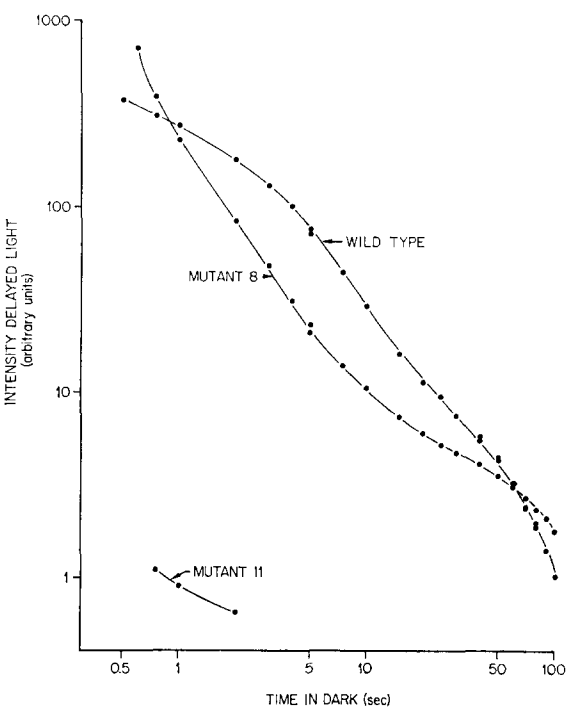

Fig. 3. Dark-decay curves of delayed light emission in the second time range. Data were taken with the single-exposure shutter apparatus. The emission from mutant 8 had decay kinetics which were different from the wild-type, and from 1 to 50 sec mutant 8 emitted less light than the wild-type. The emission from mutant 11 was barely detectable, and was about 300 times less intense than that from the wild-type for the few points which were obtained.

similar to that found with Chlorella and other plants. Mutant 8, in which photoreaction I was blocked, emitted higher intensities of delayed light than the wild-type, and the kinetics of decay were slow compared with the wild-type. At 1 msec, the emission from mutant 8 was three times as intense as the wild-type emission. At 80 msec, mutant 8 emitted more than 50 times the intensity emitted by the wild-type.

Fig. 3 shows the delayed light emission from 0.7 to 100 sec from the three strains of Scenedesmus, as measured by the single-exposure shutter apparatus. The delayed light emitted by mutant 11 was detectable only from 0.7 to 2.0 sec, and was about 300 times less intense than the wild-type emission. At 0.7 sec the emission from mutant 8 was higher than that from the wild-type, but the decay kinetics were fast compared to the wild-type. Thus, at longer times mutant 8 emitted less delayed light than the wild-type, out to about 50 sec.

TABLE II

INTENSITY OF DELAYED LIGHT, AVERAGED FROM 0.75 TO 4.2 MSEC AFTER EXCITATION, DURING STEADY-STATE PHOTOSYNTHESIS

Illumination 5 min.

	Arbitrary units of intensity
Light leak	1.4
Wild-type	16 200
Mutant 8	71 000
Mutant 11	64

Table II gives our most sensitive comparison of the relative intensities of delayed light emission from the three strains of algae: the intensity of emission averaged from 0.75 to 4.2 msec after excitation, as measured by the phosphoroscope–electrometer apparatus when the cells received 250 flashes of exciting light per sec. Mutant 11 emitted 250 times less delayed light than the wild-type, whereas mutant 8 emitted about four times more delayed light than the wild-type. The delayed light emission from all of these algal strains was destroyed by heating to 95° for 10 min.

TABLE III

EFFECT OF RED TRANSMITTING FILTERS ON DELAYED LIGHT EMISSION, AVERAGED FROM 0.75 TO 4.2 MSEC AFTER EXCITATION, DURING STEADY-STATE PHOTOSYNTHESIS

Illumination 3 min.

Corning glass filter number	*Cut-off wavelength (50% transmission) (Å)*	*Signal with filter* / Signal without filter*		
		Wild-type	*Mutant 8*	*Mutant 11*
CS 2-61	6200	0.90 ± 0.04	0.89 ± 0.03	0.89 ± 0.03
CS 2-64	6700	0.86 ± 0.05	0.88 ± 0.03	0.90 ± 0.04
CS 7-59 + CS 3-69	7200	0.35 ± 0.05	0.37 ± 0.02	0.37 ± 0.03
CS 7-69	7500	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.02

* Average of six observations; 95% confidence limits were calculated from 5 degrees of freedom.

With this sensitive phosphoroscope–electrometer method it was possible to make a crude comparison of the emission spectra of the three algal strains. This was done by inserting various filters between the cells and the photomultiplier. Each filter had a rather sharp transition from 0 % transmission of shorter wavelengths to about 85 % transmission of longer wavelengths. This transition occurred at different wavelengths in the various filters, so that various portions of the emission of the cells were transmitted. The ratio of the signal with the filter to the signal without the filter measures the fraction of the total emission in the wavelength region transmitted by the particular filter. Table III gives such ratios for the three algal strains, which were tested with four different filters. The 50 % transmission point of each filter is given, and comparison of the three strains shows that there were no significant differences in the ratios found for any individual filter. Within the accuracy of this measurement, there was no difference in the emission spectrum of the wild-type, mutant 8, or mutant 11.

DISCUSSION

Identification of photoreaction II as the delayed light emitter

If we accept the HILL AND BENDALL two-photoreaction hypothesis[17,18], then the pyridine nucleotide-reducing photoreaction (photoreaction I) is nonfunctional in mutant 8, and the oxygen-evolving photoreaction (photoreaction II) is nonfunctional in mutant 11 (Table I). Our hypothesis was that no delayed light could be emitted by the nonfunctional photoreaction of each mutant alga. This hypothesis followed from the earlier observation that delayed light emission from a photosynthetic bacterium (in which the wild-type apparently has only one photoreaction) was dependent on the presence of a functional bacteriochlorophyll photoreaction[28]. The hypothesis was justified in the present experiments, since mutant 11 emitted far less delayed light than the wild-type (Table II, Fig. 3). This result indicates that nearly all delayed light in the time-range 10^{-3} sec to 10^{2} sec is emitted due to energy storage by the oxygen-evolving photoreaction, photoreaction II.

The idea that delayed light emission is mainly from the oxygen-evolving photoreaction is not new; GOEDHEER pointed out that this hypothesis would explain the "luminescence quenching" effect of 6800-Å exciting light (absorbed preferentially by photoreaction I) on the 0.1-sec delayed light emission[8,9]. Although this observation by itself is insufficient to prove the hypothesis[7], our present observations indicate that GOEDHEER's conclusion was correct: the oxygen-evolving photoreaction emits nearly all of the delayed light. This conclusion is also consistent with the observation that differential excitation of the two photoreactions does not change the emission spectrum of the delayed light[29].

With the use of various filters we found no differences in the emission spectra of the three strains of Scenedesmus (Table III). Since the first excited singlet of chlorophyll is known to be the delayed light emitter in all plants which contain chlorophyll *a* (refs. 2–4), this observation indicates that all the delayed light we measured was emitted from chlorophyll. The use of filters is far too crude a method to answer the specific question of whether the weak delayed emission from mutant 11 (blocked in photoreaction II) could be ascribed to one or the other of the two photoreactions.

Evidence concerning the HILL AND BENDALL *hypothesis*

Mutant 8 (blocked in photoreaction I) emitted delayed light with decay characteristics which were different from the wild-type in both the msec and sec time ranges. This result is interpreted in the msec time range as implying that lack of function of the pyridine nucleotide-reducing photoreaction blocks electron flow from the oxygen-evolving photoreaction. Thus at dark times in the range of the turnover time of the enzyme system (10^{-2} sec in steady state)[30,31], photoreaction II of mutant 8 should store more energy than in the wild-type, and this energy should be depleted very slowly. If we take delayed light in the time range of msec as a measure of this energy storage at photoreaction II, then our data are compatible with the HILL AND BENDALL hypothesis.

At dark times much longer than the turnover time of the enzyme systems, one would expect photoreaction II of mutant 8 to have more energy stored than photoreaction II of the wild-type, assuming the same secondary reactions were present in both strains of algae. Our observations in the second time range (Fig. 3) do not confirm this prediction. The emission from mutant 8 crossed that of wild-type and remained less intense than the wild-type until about 50 sec of darkness This observation implies that during this time range lack of function of photoreaction I results in less energy being stored by photoreaction II. This effect dies away after about 50 sec, because at this time the emission from mutant 8 again becomes more intense than that from the wild-type. We conclude that energy stored in the functional photoreaction I of the wild-type may slowly migrate (taking 1–50 sec) to the reaction center of photoreaction II, where the energy may then result in delayed light emission. Because of the long times involved in this energy migration, we assume that the energy is migrating as charge rather than as electronic excitation.

The modification of the HILL AND BENDALL hypothesis in which two separate pigment systems are linked only by a single electron-transport chain is shown in Fig. 4. This modification provides only one mechanism by which energy may migrate from photoreaction I to photoreaction II: oxidizing equivalents may migrate through the electron-transport chain from photoreaction I to photoreaction II. This mechanism cannot account for our observations, since it would only reduce emission by photoreaction II of the wild-type. We therefore conclude that our delayed light results in

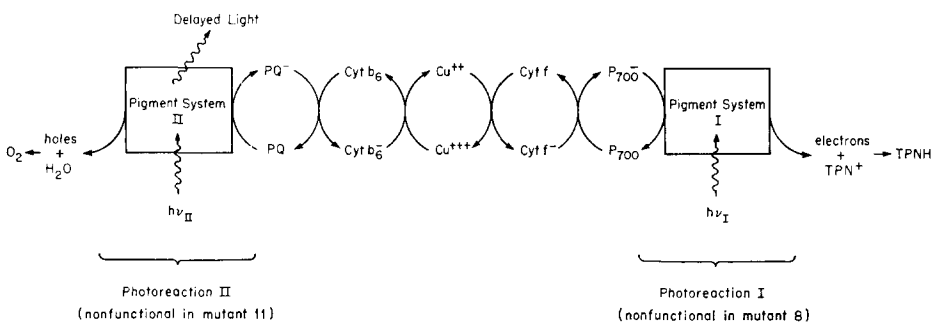

Fig. 4. Diagram of the HILL AND BENDALL two-photoreaction hypothesis. Components of the electron-transport chain have been included, although additional components may be present and although the position of certain components (such as cytochrome b_6 and plastocyanine) is by no means certain. Ferridoxin and the flavoprotein which link photoreaction I to TPN^+ reduction are not shown on the diagram. The following abbreviations are used: PQ, plastoquinone; Cyt, cytochrome; Cu, plastocyanine.

the sec time range are inconsistent with the modification which links two separate pigment systems by a single electron-transport chain.

Possible modifications of the HILL AND BENDALL *hypothesis which account for slow charge migration from photoreaction I to photoreaction II*

Fig. 5 gives schematic diagrams of three modifications of the HILL AND BENDALL hypothesis. The modification Fig. 5a shows two separate pigment systems linked by a single electron-transport chain as in Fig. 4. The other modifications (Figs. 5b and c) are consistent with the slow charge migration from photoreaction I to photoreaction II which is indicated by the present delayed light measurements in the sec time range (Fig. 3). Both modifications provide a pathway for high-energy reducing equivalents to migrate from photoreaction I to photoreaction II.

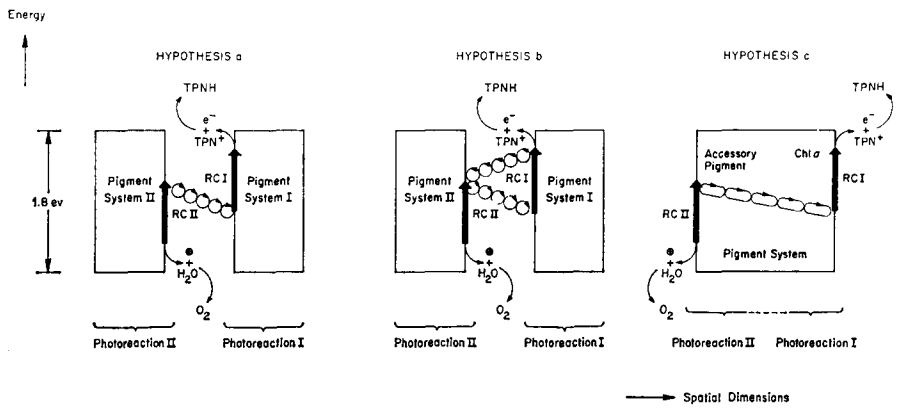

Fig. 5. Diagram of three modifications of the HILL AND BENDALL hypothesis. Modifications 5b and 5c are consistent with the slow charge migration from photoreaction I to photoreaction II which results in long-term delayed light emission from photoreaction II due to energy originally stored by photoreaction I. 1.8 eV represents the energy of the first excited singlet of chlorophyll. The heavy arrows indicate the amount of this energy actually converted to chemical potential. Energy storage is presumed to take place at the chlorophyll–enzyme interfaces of reaction centers I and II (RC I and RC II). The heavy arrows have been arbitrarily placed about midway in the energy gap between the ground state and the first excited singlet of chlorophyll, since there is no evidence to indicate their real position between the energy states of chlorophyll.

The modification Fig. 5b adds a second electron-transport chain, as suggested by ARNON[32]. This second electron-transport chain gives a mechanism by which slow charge migration resulting in delayed light could occur from photoreaction I to photoreaction II, since high-energy reducing equivalents from photoreaction I could travel slowly down this chain in the dark. At the energy storage site of photoreaction II these electrons would have some probability of producing delayed light emission. However, the existence of a second electron-transport chain, as shown in Fig. 5b, is uncertain[33–35].

The modification Fig. 5c unites the two pigment systems of Fig. 5a into a single pigment system. The single pigment system of this hypothesis provides a route for slow charge migration between photoreaction I and photoreaction II, since semiconducting properties might be expected in the highly condensed pigment system of chloroplast lamellae. In order for this hypothesis to result in two photoreactions

which may be separated by wavelength of exciting light, the composition of the single pigment system must vary in space from one reaction center to the other. EMERSON enhancement would be due to the condition that a quantum absorbed near an empty reaction center would have a greater probability of being used in photosynthesis than a quantum absorbed near a filled reaction center. This condition results from the fact that the probability of an excitation being lost as heat increases with the number of molecules over which the excitation must migrate on the average before it finds an empty reaction center. Since the highest accessory pigment concentration is near reaction center II and the highest chlorophyll *a* concentration near reaction center I, simultaneous absorption of light by both accessory pigment and chlorophyll *a* in Fig. 5c would result in a higher efficiency of photosynthesis than would absorption by either pigment alone.

The existence of a single functional pigment system would not predict any particular physical structure for the photosynthetic unit. Fig. 5c indicates the presence of complete "spillover" of excitation, but does not exclude the possibility that the two photoreactions may be situated on distinct structures. It is now clear that excitation migration may take place between different structures on which pigment molecules are placed, since phycocyanin in the blue-green algae and phycoerythrin in the red algae are both found in distinct granules attached to the membranes which contain chlorophyll[36,37]. Nevertheless, all these pigments absorb light which contributes to the photochemistry of both photoreactions[38,39].

None of the schematic hypotheses of Fig. 5 provides an explanation for our basic conclusion that nearly all of the delayed light is emitted from photoreaction II. One possible explanation has been suggested by several recent observations which indicate that a pool of electrons may be stored near the reaction center of photoreaction II, possibly in a pool of plastoquinone[40,41]. This pool is not large enough to explain a difference of at least 250 times in the intensity of delayed light emission from the two photoreactions.

Evidence for a single pigment system

A number of published observations are consistent with the hypothesis that a single inhomogeneous pigment system absorbs light for the two photoreactions, as shown in Fig. 5c.

Several observations indicate that exciting wavelengths in the region of accessory pigment absorption result in equal excitation of the two photoreactions, whereas wavelengths absorbed mainly by chlorophyll *a* result in excitation of photoreaction I alone. EMERSON AND LOUIS[42] found that the quantum efficiency of photosynthesis was practically unchanged by irradiation with various wavelengths between 5000 Å and 6800 Å. MCLEOD[43] found that the saturation rate of oxygen evolution was also nearly constant with wavelength over this range. These two observations indicate that the two photoreactions run at the same rate when excited by one of these wavelengths, in spite of the different action spectra of the two photoreactions. At least two interpretations are possible: (1) the two photoreactions are energized by separate pigment systems which absorb light in the same ratio for all wavelengths between 5000 Å and 6800 Å; or (2) excitation can "spillover" from one photoreaction to the other in order to keep the two photoreactions in pace with each other. The first interpretation is unlikely because of the requirement that different ratios of

chlorophyll *a* and accessory pigment in each pigment system be combined to give the same total absorption at all wavelengths between 5000 and 6800 Å. The second interpretation has been discussed by several authors[44,45], and CLAYTON[46] has suggested that some sort of "automatic energy switch" would be needed.

The hypothesis of Fig. 5c provides for complete "spillover", and for a kind of "automatic energy switch", in one direction. According to Fig. 5c, an excitation resulting from an absorption act near a filled reaction center II would have some probability of reaching an empty reaction center I. Thus, wavelengths absorbed by accessory pigments would supply both photoreactions with approximately the same amount of energy, regardless of which particular pigments absorbed the energy. This migration of excitation would depend on the overlap between the emission spectrum of accessory pigment and the absorption spectra of the various *in vivo* forms of chlorophyll *a*. Wavelengths absorbed only by chlorophyll *a* would have a lower probability of migrating to reaction center II because the excited singlet of chlorophyll *a* is of lower energy than that of accessory pigments. Fig. 5c therefore predicts that wavelengths absorbed only by chlorophyll *a* (wavelengths longer than 6800 Å) would excite mainly photoreaction I.

HOCH AND MARTIN[47], and SAUER and collaborators[48,49] have shown that the quantum requirement (quanta per reducing equivalent) of photoreaction I in chloroplasts decreases nearly to 1 at wavelengths longer than 6800 Å. On the other hand, the quantum requirement of photoreaction II (*cf.* ref. 50), of or both photoreactions together[47,48], increases as much as 10 times at these longer wavelengths. These results imply that wavelengths absorbed only by chlorophyll *a* result in excitation of photoreaction I. This reduction of excitation migration to reaction center II on absorption of wavelengths longer than 6800 Å is consistent with our interpretation of the single pigment system of Fig. 5c.

In the wavelength region shorter than 6800 Å photoreaction I and photoreaction II each show quantum requirements of about 2 (*cf.* refs. 49,50). This implies that half the absorbed energy of these exciting wavelengths is wasted when only one photoreaction is producing chemical potential. According to Fig. 5c, wavelengths in the range of accessory pigment absorption would be equally divided between the two reaction centers, resulting in wastage of half the energy when only one reaction center produced chemical potential. The various quantum-efficiency measurements are thus consistent with the presence of a single pigment system.

The single pigment system modification of Fig. 5c accommodates the action spectra data for each photoreaction more easily than the separate pigment system modifications. The hypotheses of Figs. 5a and 5b can account for the action spectra of the individual photoreactions only by assigning mixtures of the bulk pigments to each separate pigment system. In a red alga, DUYSENS AND AMESZ[38] found that the pigment system which would activate photoreaction I would have to contain equal amounts of chlorophyll *a* and phycoerythrin, whereas the pigment system of photoreaction II would vary between 1/7 and 1/3 chlorophyll *a* to phycoerythrin. This type of result seems best accounted for in terms of a single inhomogeneous pigment system.

The modification of Fig. 5c also suggests that low-temperature fluorescence emission spectra might show up to three peaks at wavelengths longer than 6700 Å. These peaks would correspond to the lowest energy excited singlets of chlorophyll

in the single bulk pigment system and in the two reaction centers. On a similar argument, the schemes of Figs. 5a and 5b would both be expected to show either two or four peaks in the low-temperature fluorescence emission spectra, depending on whether both the bulk pigments and the reaction centers emit. Several authors have studied the low-temperature fluorescence spectra of algae and vascular plants[51–54]. Although the exact shape of the spectra is strongly dependent on the way in which the cells are frozen, it now seems clear that three different peaks (or shoulders) are present[52,53]. The single pigment system modification of Fig. 5c is the only one on which these results are easily understandable.

The possibility that charge migration through the pigments may explain our observations in the time range of 1–50 sec should not be overlooked. Dried chloroplasts, and chloroplast pigments, have been shown to have the electrical properties of semiconductors[55,56]. Living plants show thermoluminescence[55]. The long lifetime of the delayed light decay itself (Figs. 2 and 3) is best understood in terms of charge migration[7]. Nevertheless, we do not know whether the slow migration of reducing equivalents, on which we have based our interpretation of the long-term delayed light, plays any physiological role in photosynthesis.

ARNOLD[57] has suggested an electron-hole picture of photosynthesis in which a single pigment system, with separate reaction centers for oxidizing and reducing power, can be shown to account for the apparently conflicting observations of different yields and lifetimes of fluorescence from chlorophyll *in vivo*. Since ARNOLD's picture provides only a single reaction center for electrons and only a single one for holes, the picture may be taken as a detailed hypothesis for one of the reaction centers of Fig. 5c, with associated energy-harvesting pigment molecules. If we accept the various evidence which indicates that both fluorescence and delayed light are emitted mainly by photoreaction II, then ARNOLD's picture represents a possible mechanism by which charge is handled by the reaction center of photoreaction II.

Our delayed light data in the time range of seconds can be explained either by addition of a second electron-transport chain, or by the presence of a single pigment system. The two hypotheses are not mutally exclusive. Since the latter hypothesis explains several other observations, we feel that it should be considered as a real possibility. In particular, this hypothesis explains how efficient spillover may occur in one direction (photoreaction II to photoreaction I) while inefficient spillover takes place in the other direction (photoreaction I to photoreaction II). This hypothesis, that a single inhomogeneous pigment system may absorb energy for both photoreactions of photosynthesis, does not alter the usefulness of the two-photoreaction picture of non-cyclic electron flow in photosynthesis, since it is clear that light can provide energy to run two different chemical reactions.

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