Fluorescence News

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CONTENTS

CHLOROPHYLL *a* FLUORESCENCE IN THE STUDY OF PHOTOSYNTHESIS

Govindjee and P. Mohanty

Dr. Govindjee, Professor of Botony and Biophysics, University of Illinois, began investigations of photosynthetic light-reactions in 1956. Results of his research include discovery of the quenching of chlorophyll a fluorescence by far-red light in living algae cells, the different photochemical activities of forms of chlorophyll a in photosynthesis and the existence of a new pigment absorbing at 750 nm in blue-green algae. Results of his extensive measurements of the emission and excitation spectra of chlorophyll a fluorescence in algae and chloroplasts at temperatures down to −268.6°C permitted the composition of two pigment systems in photosynthesis, along with their spectroscopic properties to be defined. His investigation into the relationship of chlorophyll a fluorescence to the photochemical and photophysical process yielded predictions of relationships between the fluorescence yields of chlorophyll a and other biochemical events in photosynthesis, particularly the "conformational" changes in the chloroplast lamellae. Results of Dr. Govindjee's investigation of the mechanism of excitation energy transfer in photosynthesis suggest that Förster's "slow" mechanism of energy transfer operates in photosynthetic systems.

Dr. Govindjee is also active in investigations of delayed light emission, thermoluminescence and fluorescence transients in intact algae, chloroplasts and sub-chloroplast fragments.

Mr. Prasanna Kumar Mohanty, Research Assistant, Department of Botany, University of Illinois, with Dr. John C. Munday defined time dependence in relation to quenching of chlorophyll a fluorescence by far-red light. Collaborating with Dr. Ted Mar, he demonstrated that hydroxyalmine feeds electrons to intact cells of red algae, and hydroxylamine feeds to the "energy trap" of photosynthesis. Presently, Mr. Mohanty is completing work on his Ph.D. in plant physiology.

In green plants, chlorophyll *a* and other accessory plant pigments absorb solar radiation, and sensitize two different photoreactions of photosynthesis.[1,2] There are two photochemical systems in green plants, labeled I and II. Each photochemical system consists of a bed of harvesting pigments that feed their absorbed quanta to a photochemical conversion center where the primary oxidation reduction reaction occurs.

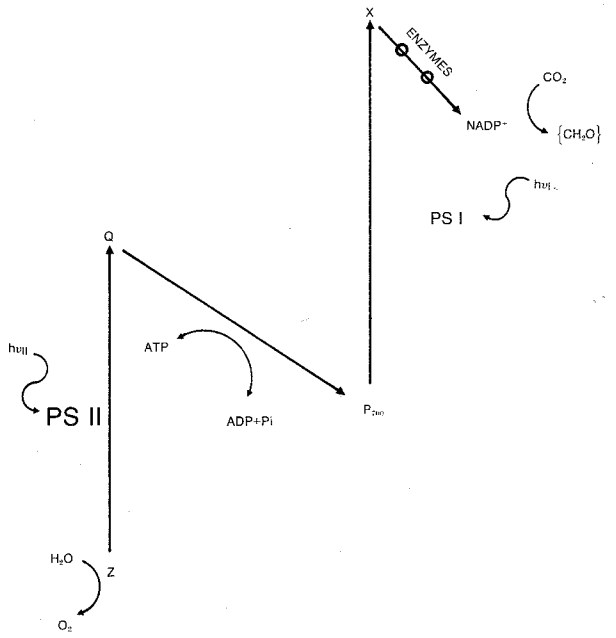

Fig. 1. Two light reactions (I and II) in Photosynthesis. Z = electron donor of System II; Q = electron acceptor of System II; P_{700} = electron donor of System I; X = electron acceptor of System I.

At the reaction center of Photosystem I (PS I), a low potential acceptor (X) is reduced (Figure 1). This reduced acceptor, in a series of enzymic reactions, reduces $NADP^+$ (nicotinamide adenine dinucleotide phosphate). The NADPH thus formed is used to reduce phosphoglyceric acid allowing the fixation of CO_2, the production of carbohydrates and many other carbon compounds that are essential for living matter.

Photosystem II (PS II) evolves O_2 from water and provides electrons to the oxidized reaction center of PS I. During the down-hill flow of electrons from the PS II electron acceptor to the PS I conversion center, some energy is conserved in ATP (adenosine triphosphate) molecules — the biological energy currency. Much of our present knowledge of the nature of the two photosystems and of the early events of photosynthesis comes from study of chlorophyll (Chl) fluorescence both *in vitro* and *in vivo*. This and other spectroscopic techniques have greatly contributed to our understanding of the mechanism of photosynthesis.

It is known that PS II emits from 3 to 6% of its absorbed energy as fluorescence. Unlike PS II, PS I is weakly fluorescent at room temperature. The reason for such a difference has not been completely explored. (Perhaps it is due to the presence of a long-wave form of Chl *a* that is very weakly fluorescent.)

Upon absorption of a quantum of light, the reaction center (or the trap) of PS I is oxidized and becomes colorless. It shows a maximal absorbance change at 700 nm, and thus it is designated P700.[3] Until recently, the existence of a similar trap for PS II was indirectly deduced from fluorescence measurements. It was argued that at high intensity of excitation, photosynthesis will

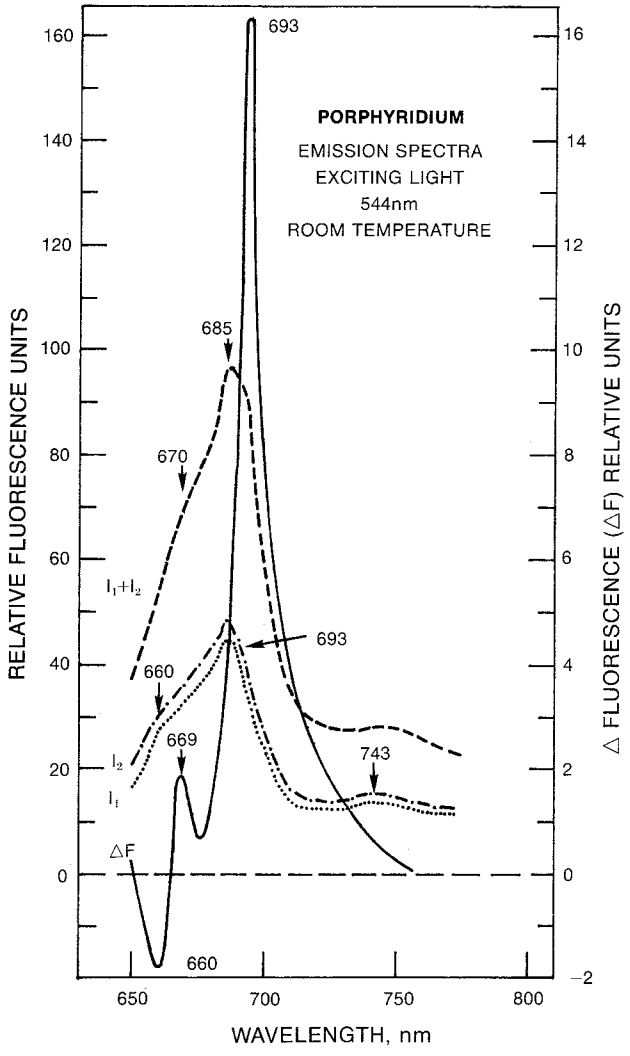

Fig. 2. Emission spectra of a red alga Porphyridium, obtained on "low" (dots, I_1; dashes and dots, I_2) and "high" intensity (dashes, $I_1 + I_2$) of 544 nm exciting light.[4, 5] The difference (solid curve) is shown on a scale enlarged ten times.

saturate, and most of the traps will remain closed. Under such a condition the closed PS II traps will also emit some fluorescence. Indeed, a shoulder at 693 nm, in addition to the main emission band at 685 nm, was observed in a red alga *Porphyridium cruentum* (Figure 2).[4] This expectation was also proven to be correct from the measurements of fluorescence emission at liquid nitrogen temperature. A distinct emission band at 696 nm, observed in several algae, is believed to originate from pigments associated with PS II traps (Figure 3).[5] Very recently, an absorbance change due to PS II traps has been detected at about 682 nm through the use of repetitive flash techniques.[6]

When all the traps of PS II are open, the photochemistry is high and fluorescence is low, and when the traps are closed, photochemistry is low and fluorescence is high. The electron acceptor of PS II is designated as Q, the quencher of fluorescence; it is a quencher in the oxidized but not in the reduced state.[7] This assignment agrees with the above observation. The light reaction II reduces Q to Q^-, and fluorescence goes up; if light I is added, Q^- is oxidized back to Q, and fluorescence is quenched. The antagonistic effect of light I on the fluorescence yield of light II is a supporting proof for the series model depicted in Figure 1.[7, 8, 9]

Reduction of Q and its reoxidation causes characteristic changes of fluorescence yield with the time of illumination (the fluorescence transient). This variation is also called the Kautsky effect.[10] Figure 4 shows a fast fluorescence transient of a green alga *Chlorella pyrenoidosa* when excited by strong system II light. Analysis of this transient has been a powerful tool in the study of the mechanism of photosynthesis.[11]

The composition of the two pigment systems (Figure 5) was obtained from the measurements of the excitation and emission spectra of Chl *a* fluorescence in whole cells and in the pigment systems separated by physico-chemical methods.[12] Experiments of Briantais, Broadman, et al, and Cho and Govindjee show that both Chl *b* and Chl *a* are present in both the systems.[13, 14, 15] System I, however, contains, in addition, a long wave form of Chl *a* which fluoresces at 710-720 nm at liquid nitrogen temperature (see Figure 3).[15, 16]

In green plants, energy transfer from the accessory pigment Chl *b* to Chl *a* has been extensively studied by the technique of sensitized fluorescence.[17] This transfer is almost 100%. However, carotenoids (with the exception of fucoxanthol) transfer only 40-50% of their absorbed quanta to Chl *a*. In blue-green and red algae where Chl *b* is replaced by phycobilins, the efficiency of energy transfer is also high — in the neighborhood of 80%. These pigments feed their absorbed photons to Chl *a*, which in turn feeds to a reaction center. Thus, these pigments act as a lens collimating the energy at the photochemical conversion center. This is the physical picture of a photosynthetic unit.[1, 2]

The mechanism of energy transfer from Chl *b* to Chl *a* is believed to be that of resonance energy migration. Förster has shown that when the fluorescence emission spectrum of a donor (like Chl *b*) overlaps the absorption

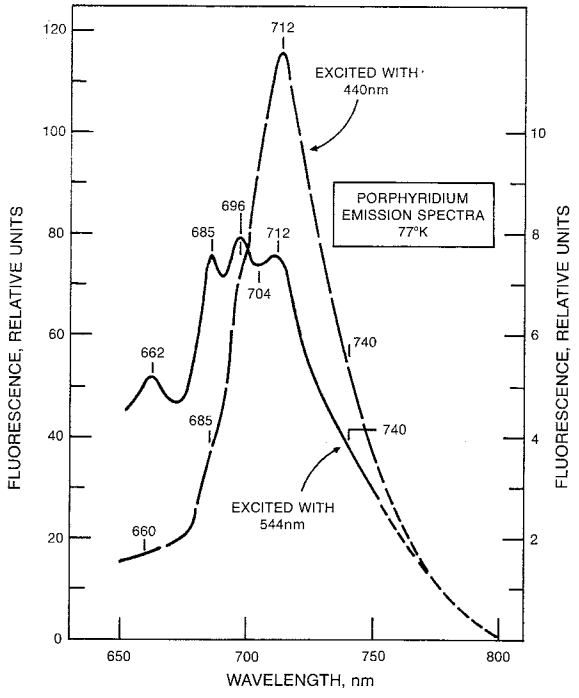

Fig. 3. Emission spectra of Porphyridium at 77° K. Solid curve: excitation was 440 nm (absorbed in Chlorophyll a, System I); dashed curve: λ excitation was 554 nm (absorbed in a phycobilin phycoerythrin, System II).[5]

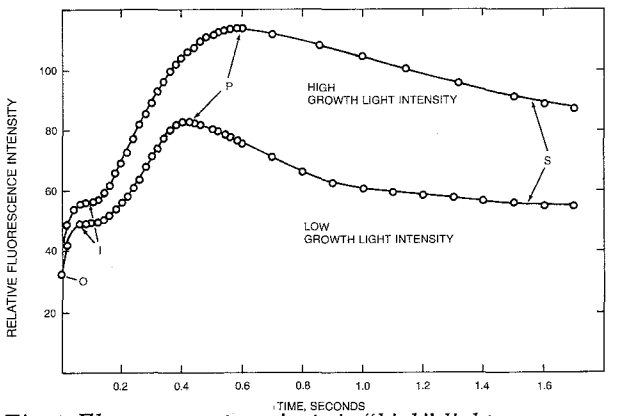

Fig. 4. Fluorescence transients in "high"-light grown and "low" light grown green alga Chlorella pyrenoidosa. *The transients were normalized at O; O I P and S (arbitrary labels) designate the various parts of the transient. (After J. C. Munday Jr., PhD. thesis, U. Ill. 1968.)*

of an acceptor (as Chl *a*), resonance transfer of energy occurs.[18] This, however, has been questioned by the observation of a lack of temperature dependence on the efficiency of energy transfer. The resonance transfer seems to be the most likely mechanism of transfer from phycocyanin (one of the phycobilins) to Chl *a*, since temperature dependence for this transfer has been demonstrated.[19]

Evidence for the existence of energy migration among

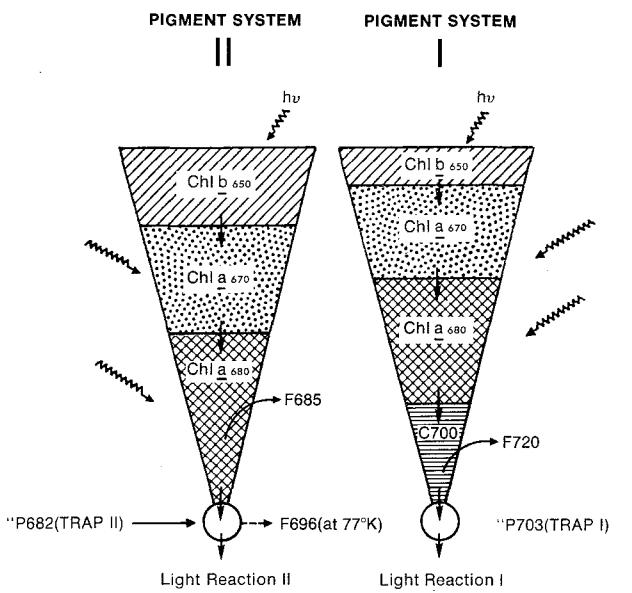

Fig. 5. Working hypothesis for the distribution of chlorophylls in two pigment systems in higher plants and green algae. Both systems may contain Chl a and Chl b650 in different proportions. (In red and blue-green algae, phycobilins replace Chl b.) This suggests the long-wave form of Chl a (C700) is present in pigment system I only. The two "bulk" chlorophylls (Chl a670 and Chl a680) are almost equal in both systems. (In the red and blue-green, a larger proportion of Chl a is in pigment system I.) The energy trap (TRAP I) of system I is P700, and TRAP II is P682. The new emission band appearing at 696 nm (F696) when plants are cooled to 77°K originates in TRAP II. At room temperature, most of the main fluorescence band at 685 nm (and its 740 nm "satellite") originates from the pigment system II; the band at 720 nm originates preferentially in pigment system I. (After Govindjee, Papageorgiou and Rabinowitch, 1967, see recent publications.)

like molecules of Chl *a* has been deduced fom the extensive depolarization of fluorescence. The mechanism of this migration has been recently investigated in our laboratory at Urbana.[20] Addition of a photosynthetic poison DCMU (3,(3,4 dichlorophenyl),1,1 dimethyl urea) to algal suspensions increases the extent of the depolarization, and thus decreases the polarization (p) of fluorescence. This observation, with certain assumptions, may suggest that the pace of energy transfer among Chl *a* molecules of system II is of the "slow" Förster type. Addition of DCMU prevents the excitation from being trapped and increases the probability of its visit to many more Chl *a* molecules, giving extensive depolarization of fluorescence.

When the excitation encounters a closed trap, it may "search" for another reaction center. Evidence for such an intra-unit energy migration among the system II units was obtained by Joliot and Joliot from the observation of a lack of proportionality between the photochemical rate of oxygen evolution and the fraction of open traps.[21] This observation suggests that the PS II reaction centers are embedded in a "lake" of pigments and are not isolated discrete units. The "lake" model of PS II units predicts a linear dependence of the life time of fluores-

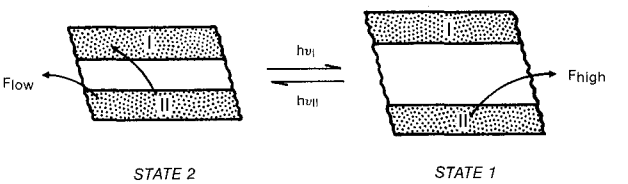

Fig. 6. Hypothetical picture of the two "conformational states" of chloroplast membranes (see text and ref. 25).

cence on its quantum yield inasmuch as most of the fluorescence comes from PS II. This prediction was proven to be valid by the measurements of Tumerman and Sorokin in U.S.S.R. and of Briantais et al in our laboratory.[22, 23] The lifetime of fluorescence increased from a low value of 0.6 nanoseconds to 2.0 nanoseconds.

In isolated chloroplasts, the rate of PS I photochemical reaction was found to increase proportionately with the amount of open traps. It was argued that PS I has a different photon trapping mechanism. There are special Chl *a* molecules near the trap called C_{700} and these prevent the exit of absorbed quantum from one unit, and its subsequent search for open traps. Evidence of intra-unit transfer among PS I units, however, has been found in some algae.[24] We have not decided whether the system I units are arranged as "lakes" or as isolated "puddles".

It has also been suggested that energy absorbed by PS II units, if not utilized, may spill over to PS I. Controversial results exist in the literature as to whether "spillover" occurs. In recent years, a new picture has begun to emerge from studies in various laboratories (including ours). These reveal that the degree of "spillover" of quanta depends on the conformational status of the membranes (thylakoids) that bear the two pigment systems. A pictorial description of such conformational changes is given in Figure 6.

In this model, a massive "spillover" is facilitated when the two pigment systems are close to each other. When the two photosystems are close, excessive "spillover" from PS II to PS I quenches the fluorescence yield of PS II, inasmuch as PS I is weakly fluorescent at room temperature. Thus, there is additional quenching superimposed on the quenching by the quencher Q. Recent studies further

show that these conformational changes are regulated by ionic environment of the chloroplast and its embedding cytoplasm.[25] In chloroplasts, mono- and di-valent cations are shown to alter conformational state of chloroplasts from one state (I) to another (II).[26] A relatively easy method to detect these two hypothetical states of conformation is to freeze the sample under the condition of one state, and then measure the fluorescence emission spectrum.

Thus, it seems that the study of fluorescence is not only indicative of the photochemical process of photosynthesis, but also of the entire physiology of the cell.

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TRIGLYCERIDES*
(Semi-Automated)

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Principle

The lipids in plasma are extracted in isopropanol containing a mixture of Zeolite, Lloyd's reagent and Van Slykes copper lime mixture. Each of these components removes phospholipids, bilirubin, chromogens and glucose respectively. In the Technicon AutoAnalyzer, the sample stream and alcoholic-KOH are mixed, heated to 50° C, which saponifies triglycerides to glycerol. The glycerol is then oxidized by periodate to formaldehyde which forms a Hantzsch condensation reaction with acetylacetone and ammonia, yielding a fluorescent product 3, 5 diacetyl, 1, 4-dihydrolutidine. The fluorescent compound is then measured.

* *Combined Methods of Kessler and Noble*

Reagents

1. Zeolite Mixture (Nobles modification)
   (a.) Zeolite — (Taylor Catalog No. 285) — 200gm.
   Baltimore 4, Maryland
   (b.) Lloyds reagent — (Harleco Catalog No. 5895) — 20gm.
   (Hydrated Aluminum Silicate)
   Harleco, Philadelphia, Pennsylvania
   (c.) Copper Sulfate — (Fisher Catalog No. C-493) — 20gm.
   (d.) Calcium Hydroxide — (Fisher Catalog No. C-97) — 40gm.
   Grind and mix in a blender, and heat to 110° C in an oven overnight. Cap while still hot, and