Chapter 15

Fluorescence and the Two Pigment Systems

Preceding chapters dealt with experimental evidence underlying the concept of two primary photochemical reactions sensitized by two separate pigment systems, being involved in photosynthesis. Further arguments supporting this concept, and supplying additional details, can be derived from the study of chlorophyll fluorescence in vivo.

Fluorescence measurements permit practically instantaneous nondestructive monitoring of rapidly changing chemical systems that involve fluorescent reactants, intermediates, or products. Photosynthesis is such a process. Chlorophyll is fluorescent in vivo as well as in vitro, and so are the phycobilins. For reasons to be explained later, we are mainly interested in the fluorescence of chlorophyll.

The fluorescence yield of chlorophyll *a* (ratio of the number of quanta emitted and the number of quanta absorbed) is much lower in vivo than in vitro (3–6% in vivo, against 30% in vitro). For a time, the very existence of chlorophyll fluorescence in plants was disputed. However, modern experimental techniques make it possible not only to demonstrate the existance, and measure precisely the intensity of plant fluorescence, but also to determine its spectral composition and the action spectrum for its excitation. A scheme of such an instrument is shown in Fig. 15.1.

It appears that several forms of chlorophyll *a* contribute to fluores-

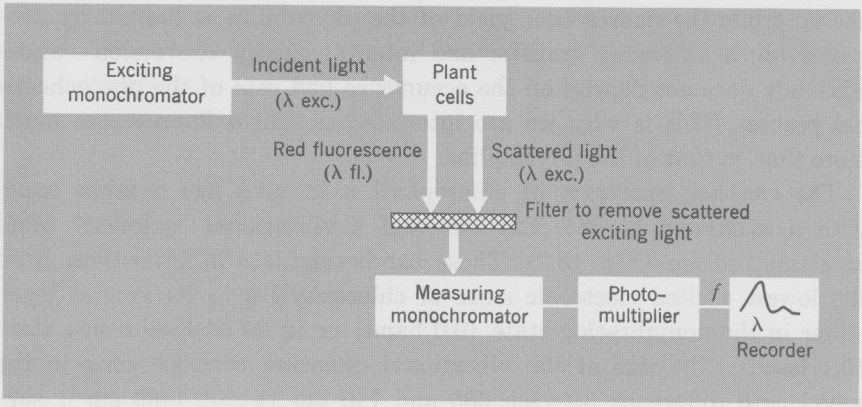

FIG. 15.1 Schematic diagram of a spectrofluorometer. A common modification of this setup is to collect fluorescence from the same side where the incident light hits the cuvette containing plant cells; this is done to reduce the reabsorption of fluorescence.

cence in vivo (as they were shown in Chapters 9 and 13 to contribute to light absorption). Each has its own characteristic emission spectrum. The yield of fluorescence of these components is quite different; and their excitation spectra differ, too. They have a different dependence on intensity of illumination, and change differently during the induction period.

We have already discussed some applications of fluorescence studies in Chapter 12, when talking of excitation energy transfer between pigments in vivo. These studies have revealed that excitation energy is transferred from all accessory pigments to chlorophyll *a*. The transfer from the phycobilins to chlorophyll requires a few nanoseconds; this gives enough time for phycoerythrin and phycocyanin to emit some fluorescence. Energy transfer from chlorophyll *b* to cholorophyll *a* is so fast that no Chl *b* fluorescence has been observed in plants.

The yield of fluorescence of the phycobilins proves to be independent of the intensity of illumination, presence of poisons, and other factors affecting the rate of photosynthesis. The yield of fluorescence of chlorophyll *a*, on the other hand, in addition to being much smaller than in solution, is significantly affected by these factors. Clearly, chlorophyll *a* fluorescence competes with the use of excitation energy for photosyn-

thesis; while the fluorescence yield of the phycobilins is limited by processes—such as energy transfer and internal energy conversion—whose efficiency does not depend on the occurrence and rate of the photochemical process. This is why we are interested in Chl *a* fluorescence much more than in that of the phycobilins.

The *emission spectrum* of chlorophyll *a* in vivo has a main band, with a maximum at 685(±2) nm, and a vibrational "satellite" band at about 740 nm (Fig. 15.2). These bands originate in transitions from the lowest excited electronic state of chlorophyll *a* to its ground level, either in the nonvibrating state (0,0 band) or in its first vibrating state (0,1 band). The size of the vibrational quantum corresponding to the wavelength difference between 685 and 740 nm (about 1460 cm^{-1}) sug-

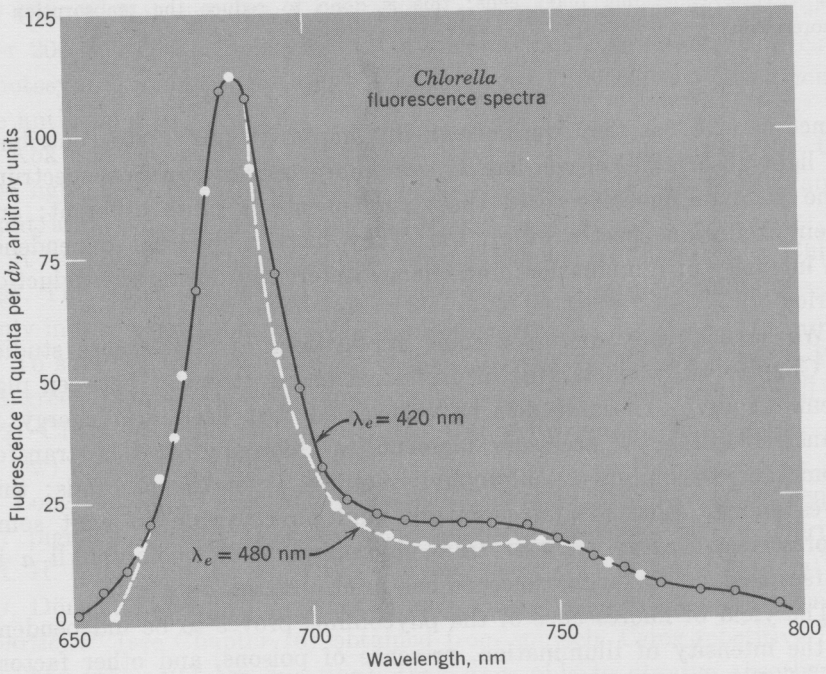

FIG. 15.2 Fluorescence spectra of *Chlorella pyrenoidosa* excited at 420 nm (λ_e = 420) and 480 nm (λ_e = 480), showing increased excitation of F720 by light preferentially absorbed in Chl *a* (420 nm) compared to light preferentially absorbed in Chl *b* (480 nm). (Duysens, 1952; confirmed in spinach chloroplasts by Govindjee and L. Yang, 1966.)

gests that the vibration coupled with the electronic transition may be a C—H "bond deformation" vibration—a fact of some interest in relation to the mechanism by which excitation energy is used for the primary photochemical process.

Excitation in the blue-violet (Soret) band leads to a higher, "blue," excited electronic state; but it is quickly followed (in vivo as well as in vitro), by radiationless internal conversion into the "red" excited state, with the loss of about one-third of excitation energy. This transition is so fast ($<10^{-10}$ sec), that no emission originating in the "blue" state has been observed.

E. C. Wassink (in Holland), and James Franck (in Chicago), had observed a striking increase (approximate doubling) of the fluorescence yield of chlorophyll a in vivo with increasing intensity of illumination, apparently associated with the light saturation of photosynthesis. Hans Kautsky and co-workers (in Germany) first described another striking phenomenon—changes in fluorescence intensity that occur when cells are suddenly brought from darkness into light, during the so-called *induction* period of photosynthesis. Application of more sensitive instruments has revealed that both these changes affect differently the several spectroscopic components of fluorescence.

Studies in liquid nitrogen and liquid helium showed considerable increase in the yield of fluorescence at low temperatures, and appearance of new emission bands probably due to chlorophyll *a* forms not present (or nonfluorescent) at room temperature.

Studies with plane-polarized light provided some information about the spatial arrangement of chlorophyll molecules in the photosynthetic units.

We will discuss below only a few results of all these studies, most clearly related to tht two-pigment-systems hypothesis.

SPECTRUM OF CHLOROPHYLL *a* FLUORESCENCE IN VIVO

Fluorescence at Room Temperature

"STRONGLY" AND "WEAKLY" FLUORESCENT CHL *a* FORMS IN VIVO. The main emission band of Chl *a* in vivo (F685)[1] appears to contain, at

[1] We designate fluorescence bands by the letter F; absorption bands can be similarly designated by the letter A.

room temperature, a weak component, at (or beyond) 700 nm. (Its precise position is obscured by the above-mentioned vibrational subband at 740 nm.) The doublet nature of F685 is revealed, for example, by matrix analysis. Exciting fluorescence at one set of wavelengths, λ_{ex}, and measuring its intensity at another set of wavelengths, λ_{em}, a series of matrices can be constructed. If all 2×2 matrices[2] are not equal to zero, there must be, according to Gregorio Weber, at least two fluorescence emitters; if some 3×3 matrices do not disappear, there must be at least three fluorescence emitters, etc. Applying Weber's analysis to the fluorescence of spinach chloroplasts, Louisa Yang and Govindjee found that at room temperature (at least) two Chl *a* forms contribute to fluorescence. S. S. Brody obtained similar results on *Euglena*.

When *Chlorella* cells (or spinach chloroplasts) are excited at 440 nm (that is, with light absorbed preferentially by chlorophyll *a*), the long-wave component (F720) is (relatively) stronger than when 480 nm light (absorbed preferentially by chlorophyll *b*) is used for excitation (Fig. 15.2). This suggests that a larger part of F720 originates in PSI. (We have concluded before that this system contains less chlorophyll *b* than PSII.) The main band, F685, probably originates in PSII, which contains a larger part of Chl *b*.

The conclusion that Chl a_{II} (Chl *a* in system PSII) is the main source of chlorophyll *a* fluorescence in vivo is confirmed by a variety of observations. L. N. M. Duysens (in Holland) and C. Stacy French (in Stanford, California) have observed that in red algae, green light, absorbed mainly by the phycobilins, is—paradoxically!—more efficient in exciting chlorophyll *a* fluorescence than light absorbed in chlorophyll *a* itself. (These were the observations that first led Duysens to postulate the existence, in red algae, of two forms of chlorophyll *a*, the more strongly fluorescent one being preferentially associated with the phycobilins.) The same is true of blue-green algae (Fig. 15.3). (It was suggested in Chapter 13 that phycobilins are more abundant in PSII than in PSI!)

We can thus assume that the "fluorescent" form of Chl *a* belongs

[2] A 2×2 matrix consists of four numbers—in our case, of four intensity values, $I\lambda_{ex}\lambda_{em}$, corresponding to excitation wavelengths λ_{ex_1} and λ_{ex_2}, and emission wavelengths λ_{em_1} and λ_{em_2}. The value of the matrix is the difference $I(\lambda_{em_1}\lambda_{em_1})I(\lambda_{ex_2}\lambda_{em_2}) - I(\lambda_{ex_2}\lambda_{em_1})I(\lambda_{ex_1}\lambda_{em_2})$.

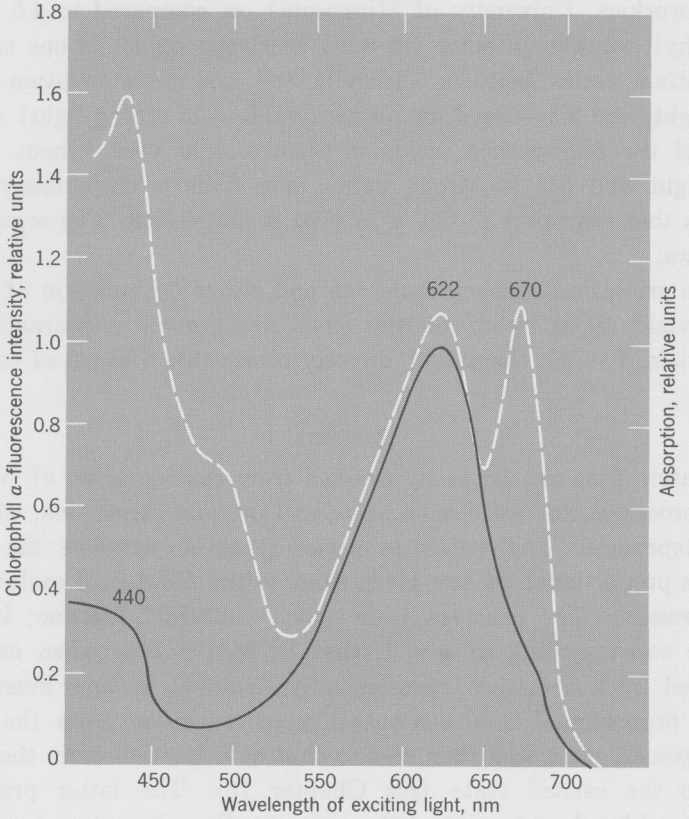

FIG. 15.3 Fluorescence excitation spectrum of blue-green algae (*Anacystis nidulans*) measured at 745 nm, compared to absorption spectrum (dashed curve), indicating more efficient excitation of Chl *a* fluorescence by light absorbed in phycocyanin (at 620 nm) than by light absorbed by Chl *a* itself (at 420 and 670 nm). (G. Papageorgiou and Govindjee, 1967.)

to PSII, and the "nonfluorescent" (or, more exactly, less fluorescent form), to PSI.

Another indication of the existence of two forms of Chl *a*, with different fluorescence yields, was obtained from measurements of the average lifetime, τ of chlorophyll *a* fluorescence. It was found to be about 0.7 nsec in weak light (W. J. Nicholson and J. Fortoul, I.B.M., New York; G. Singhal, University of Illinois)*, and 2 nsec in strong light (R. Lumry

* About 0.6 nsec according to W. J. Nicholson and J. Fortoul (1967) and down to 0.4 nsec, according to A. Muller, R. Lumry and M. S. Walker (1969).

and co-workers, University of Minnesota), as compared to 4.5 nsec. in chlorophyll solution in ether (in weak or strong light). If one compares the lifetime ratios between Chlorella cell and ether solution (6.5 in weak light, and 2.5—based on 1.3 nsec value— in strong light) with the ratios of the fluorescence *yields* in vitro and in vivo (about 10:1 in weak light and 5:1 in strong light), one finds a discrepancy, which suggests that only part of Chl *a* in vivo is fluorescent. The argument is as follows:

If the excitation of a molecule can end either by emission of fluorescence (yield, Φ) or by an internal quenching process ("internal conversion," yield, $1 - \Phi$), the actual, directly observable lifetime of excitation will be

$$\tau = \Phi\tau_0 \tag{15.1}$$

—a relation that can be easily derived from consideration of two competing processes, fluorescence emission and internal conversion, treated as two independent "first order" processes (that is, processes the rate of which is proportional, at any given time, to the number of excited molecules present). The constant τ_0 is called "natural" lifetime; it is the lifetime corresponding to $\Phi = 1$, that is, to the case when excitation is limited by fluorescence emission only. Natural lifetime must be inversely proportional to the probability of transition from the excited to the ground state, and thus also to that of transition from the ground state to the excited state (see Chapter 10). The latter probability is measured by the intensity of the corresponding absorption band (more precisely, by the integral of the plot $\alpha = f(\lambda)$, where α is the absorption coefficient at wavelength λ, integration being extended over the whole absorption band). This integration gives, in the case of Chl *a* in vitro, $\tau_0 = 15$ nsec. Inserting this value, and the above-mentioned experimental value of τ (4.5 nsec) into Eq. 15.1, we obtain

$$\Phi = \frac{\tau}{\tau_0} = \frac{4.5}{15} = 0.3, \text{ or } 30\% \tag{15.2}$$

—in good agreement with direct determination of the fluorescence yield of Chl *a* in solution. However, no such agreement is found in the case of fluorescence in vivo. Since it is difficult to determine the integral of the absorption band in vivo, one makes, in this calculation, the plausible assumption that the natural lifetime of excited chlorophyll, τ_0, is the same in vivo as in vitro. With $\tau = 0.7$ nsec (weak light) or 1.3 nsec (strong light) one calculates from Eq. 15.2, $\Phi = \tau/\tau_0 = 5\%$ (weak light)

or 9% (strong light), while direct measurements of the fluorescence yield in vivo give 3% in weak light and 6% in strong light. This discrepancy can be explained by assuming that a significant fraction of Chl *a* in vivo is nonfluorescent (or only weakly fluorescent). In this case, Φ should be determined by referring the emission not to *total* chlorophyll absorption, but only to absorption by the fluorescent component. The results suggest that about one half of chlorophyll *a* in vivo is nonfluorescent, in weak as well as in strong light.

ACTION SPECTRUM OF CHL *a* FLUORESCENCE IN VIVO: THE "RED DROP." If the "weakly fluorescent" form of chlorophyll *a* in vivo absorbs relatively more strongly on the long-wave side of the red absorption band, a "red drop" can be expected in the action spectrum (the plot of the quantum yield of fluorescence, Φ, as function of wavelength of exciting light, λ_{ex}). Duysens (1952) did in fact note such a decline in green algae beyond 680 nm. Direct measurements of Φ (as well as calculations based on relationship between the absorption and the emission spectrum) made in Urbana, also indicated a drop of Φ, beginning (in *Chlorella*) at about 675–680 nm (see Fig. 15.4).

The action spectrum of chlorophyll fluorescence in red algae is

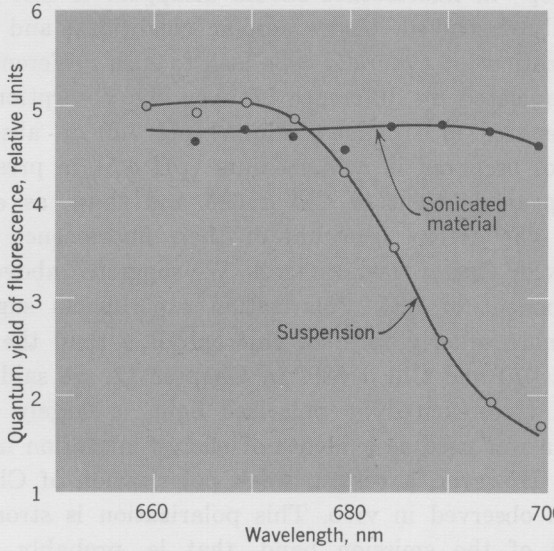

FIG. 15.4 Red drop in action spectrum of fluorescence in *Chlorella* and its disappearance in aerobic acid sonicates of *Chlorella*. (M. Das and Govindjee, 1967.)

quite different from that in green algae, because almost all Chl *a* seems to be present there in PSI. Consequently, the spectral region of predominant absorption in PSI begins, in red algae, at much shorter wavelengths than in the green ones, and the "red drop" starts correspondingly earlier, when absorption in phycocyanin ceases to be strong compared to that in Chl *a*. (This consideration applies to the action spectrum of photosynthesis as well as to that of fluorescence.)

In the red alga *Porphyridium cruentum,* and the blue-green alga *Anacystis nidulans,* the fluorescence yield is found, in fact, to decline already at 640–660 nm.

One is tempted at first to associate the "weakly fluorescent" component of Chl *a* with Chl *a* 680, as identified from absorption data (see Fig. 9.7). However, quantitative analysis argues against this identification. Instead, the "red drop" seems to be associated with a third, minor Chl *a* component present only in PSI (French's "Chl *a* 695," or Butler's "C700"), while both main forms, Chl *a* 670 *and* Chl *a* 680, present in both PSI and PSII, contribute about equally to fluorescence. The presence of Chl *a* 695 in PSI makes *all* Chl *a* in this system non- (or weakly) fluorescent, by draining and dissipating the excitation energy from the two main components.

The "red drop" in fluorescence should disappear if Chl *a* 695 could be preferentially destroyed. Under aerobic conditions, and at low pH, prolonged sonication[3] of *Chlorella* cells lead to such preferential destruction. This is indicated by difference between the absorption spectra of "sonicates" prepared in alkaline medium (pH 7.8) in absence of air, and of sonicates prepared in acid medium (pH 4.5) in presence of air. The latter appear deficient in Chl *a* 695 and show, as expected, no "red drop" in the action spectrum of their fluorescence (Fig 15.4).

POLARIZATION OF CHL *a* FLUORESCENCE. We suggested above that Chl *a* 695 is a component of PSI. Polarization experiments suggested that it may be a more orderly form of chlorophyll *a* than the main components, Chl *a* 670 and Chl *a* 680. In Chapter 12, we said that Chl *a* fluorescence in vivo, excited by polarized light, is largely depolarized; this observation was used as evidence of energy migration in the photosynthetic unit. However, a certain weak polarization of Chl *a* fluorescence has been observed in vivo. This polarization is strongest in the long-wave end of the emission band, that is, probably to emission from PSI.

[3] "Sonication" means treatment by ultrasonic waves.

INDUCTION OF CHL *a* FLUORESCENCE IN VIVO. The complexity of Chl *a* fluorescence in vivo is confirmed by its variation with time during the induction period of photosynthesis. When *Chlorella* cells are exposed to bright light after a long dark period, the fluorescence yield Φ practically instantaneously reaches a certain level [*O* in Fig. 15.5*a*]. Within a

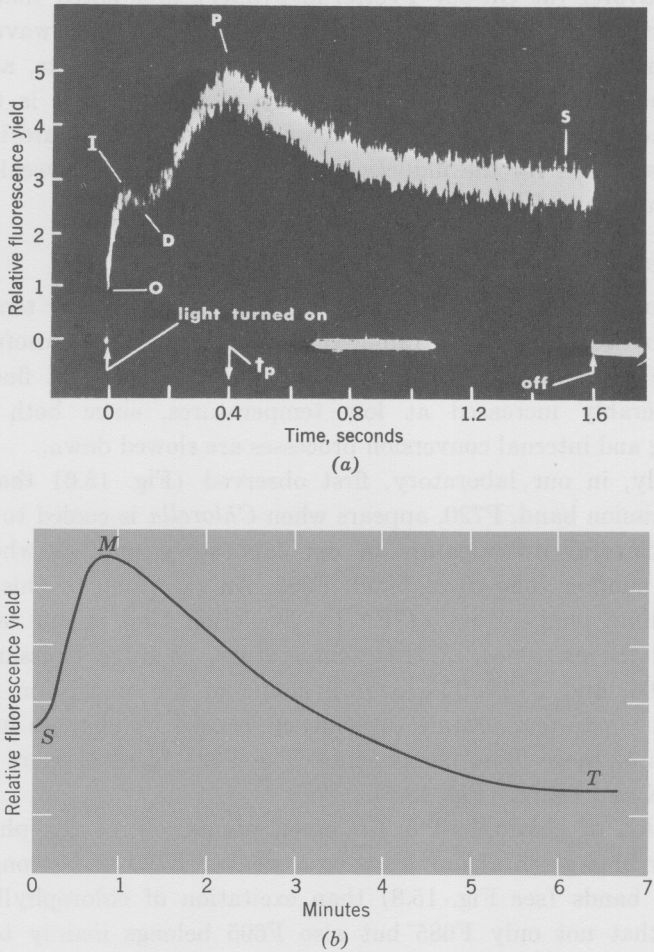

FIG. 15.5 Fluorescence induction in aerobic *Chlorella pyrenoidosa*. Wavelength of measurement: 685 nm; excitation: blue light. (*a*) Picture taken on the oscilloscope screen—up to 2 seconds. (J. C. Munday and Govindjee, 1969.) (*b*) Measurements with a Brown recorder—from 3 seconds to 6 minutes. (G. Papageorgiou and Govindjee, 1968.) For meaning of letters, see text.

few milliseconds, Φ rises to a higher level, I; it then decreases slightly to D, increases again to a peak P, reached after about 0.5 seconds, and declines to a more or less steady state S. A slow rise to a second maximum (M), follows, which takes about 40 seconds, and is followed by a decline to a terminal stationary state (T), reached after several minutes (Fig. 15.5*b*).

Jean Lavorel (at Gif-sur-Yvette, in France) first noted that at time O fluorescence contains relatively more of the weak, long-wave fluorescence band (F720) than in the peak P. These experiments, and many others, including those in our laboratory, suggest that it is the main fluorescence band at 685 nm (which we presume to originate in system II) that accounts for the induction phenomena, while the weak fluorescence from system I remains practically constant.

Fluorescence at Low Temperatures

EMISSION SPECTRA. Sharpening of emission bands at low temperature (caused by cessation of intramolecular vibrations) is a useful means to reduce their overlapping. The quantum yield of total fluorescence is considerably increased at low temperatures, since both external quenching and internal conversion processes are slowed down.

S. Brody, in our laboratory, first observed (Fig. 15.6) that a new strong emission band, F720, appears when *Chlorella* is cooled to —190°C (77°K). Several investigators in our laboratory and elsewhere have observed another long-wave band, F695. An extension of this work to liquid helium temperature (4°K) by Frederick Cho in our laboratory confirmed the existence, at that temperature, of three emission bands, F689, F698, and F725. The overall shape of the emmission spectrum exhibits a strong temperature dependence, caused by changes in relative intensities of these three components. The F698 band appears only between 4°K and 140°K (Fig. 15.7).

Excitation of chlorophyll *b* (in green plants) or of the phycobilins (in red or blue-green algae) leads to a weaker F720 and stronger F685 and F695 bands (see Fig. 15.8) than excitation of chlorophyll *a*. This suggests that not only F685 but also F695 belongs mainly to system II, while F720 belongs preferentially to system I. This is confirmed by the finding that particles (prepared by the N. K. Boardman and J. Anderson's digitonin fractionation method, see Chapter 16) which perform preferentially light reaction I, show, at 77°K, a preponderance of

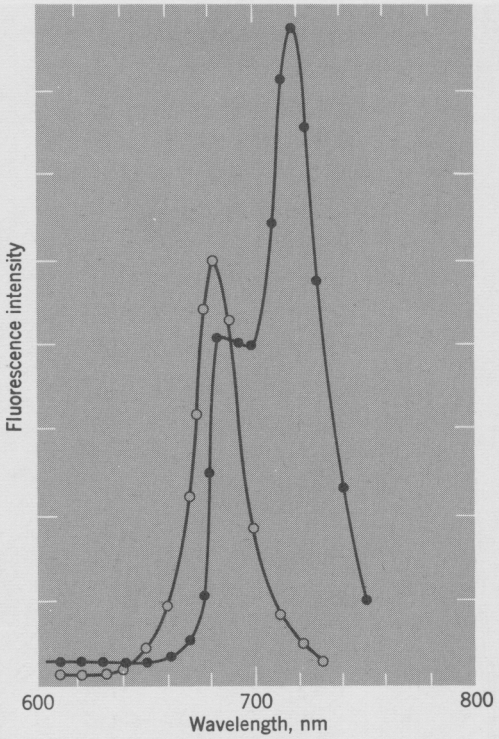

FIG. 15.6 Fluorescence spectrum of *Chlorella* at −177°C, showing a strong band at 720–725 nm (black dots); spectrum at room temperature is shown for comparison (circles). (S. Brody, 1958.)

F725, whereas particles that perform preferentially light reaction II, appear enriched in F685 and F695-emitting material.

FLUORESCENCE FROM "TRAPS"? The energy trap in pigment system II has been only recently identified by difference spectroscopy; the rate of regeneration of this trap is very fast ($\sim 10^{-4}$ sec) as opposed to that of P700 ($\sim 10^{-2}$ sec) (see Chapter 14). Cooling should favor fluorescence emission from traps, because at low temperature, energy quanta caught in a trap will find it difficult to get back into the "bulk" of the pigment before emitting fluorescence. The trap depth, estimated from the displacement of the absorption bands (for example, the shift from 680 nm, where the absorption band of the "bulk" is located, to 690 nm, where we sup-

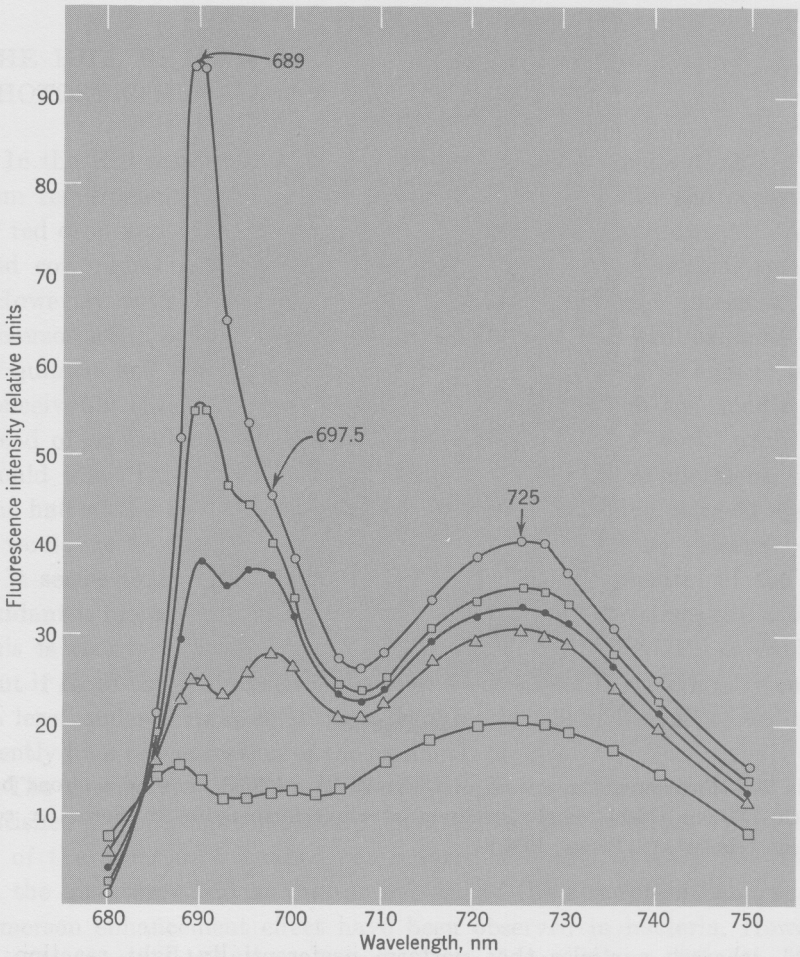

FIG. 15.7 Fluorescence spectra of *Chlorella* at several temperatures down to —269°C. (□, —196°C, lowest curve; Δ, —218°C; ●, —233°C; □, —247°C; ○,—269°C.) (After F. Cho, J. Spencer, and Govindjee, 1966.)

pose the band of the "trap" in PSII is found), is 1–2 kT at room temperature, 5–8 kT at 77°K (in liquid nitrogen) and 100–150 kT at 4°K (in liquid helium); the probability of escaping from this trap in $< 10^{-8}$ sec. (the lifetime of excitation), is very high in the first case and practically nil in the last one. The F695 band, which appears only

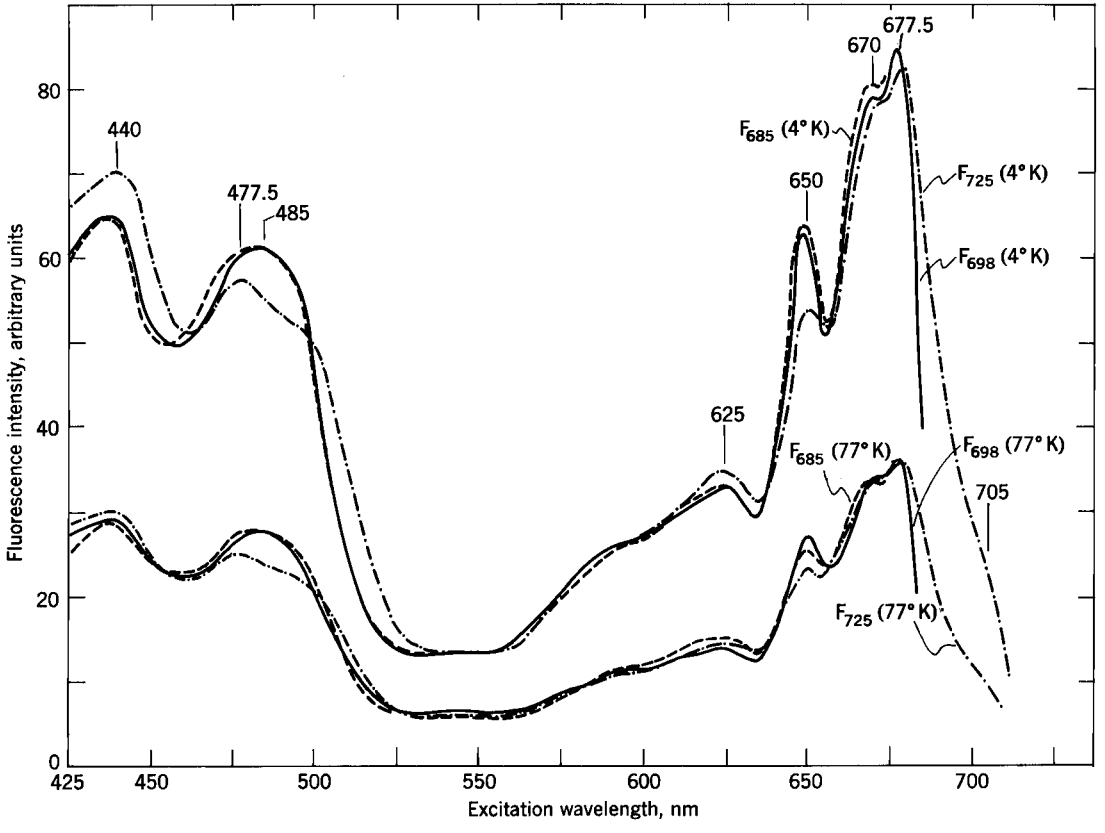

FIG. 15.8 Excitation spectra of fluorescence (for F685, F698, and F725) in *Chlorella* at 4°K (upper curves) and 77°K (lower curves). (F. Cho and Govindjee, 1969.)

below 140°K, is thus a likely candidate for fluorescence emission from the trap in system II.

ACTION SPECTRA. That the bands F685 and F698 are excited mainly by absorption in system II, and the band F725 mainly by absorption in system I, is confirmed by measurements, by F. Cho, of the excitation spectra of these bands (in *Chlorella*) at 4°K (Fig. 15.8). For example, we note that the ratio of the intensity of fluorescence excited at 440 nm (in the peak of Chl *a* absorption band) to that excited at 485 nm (in the peak of Chl *b* absorption band) is greater for F725 than for F685 and F698. Furthermore, the F725 excitation spectrum has a much less pronounced peak at 650 nm (in the peak of Chl *b* red band) than those of F685 and F698. Near identity of the excitation spectra for F685 and F698 in Fig. 15.8 confirm that both belong to the same system (system II) while the presence of a band >700 nm in the excitation spectrum of F725 confirms that the latter originates mainly in system I (probably, in Chl *a* 695).

Another interesting feature of Fig. 15.8 is the appearance, at 4°K, of two separate excitation peaks in the red, at about 670 and 680 nm. These wavelengths clearly correspond to the two absorption peaks of chlorophyll *a* in vivo identified by analysis of the red band at room temperature (see Chapter 9). Appearance of these peaks in the excitation spectra of both F725 (Chl a_I) and F698 (Chl a_{II}) confirms the suggestion that PSI as well as PSII contain both components, although apparently in somewhat different relative concentration.

DEPENDENCE OF FLUORESCENCE YIELD ON LIGHT INTENSITY

In dye *solutions* (including those of chlorophyll) the fluorescence intensity (F) is proportional, within wide limits, to the intensity of illumination (I); in other words, the *yield* of fluorescence does not depend on it. Chlorophyll *a* fluorescence in vivo shows, however, a very significant dependence of the yield on the intensity of illumination (see Fig. 15.9, where the upper curve shows the fluorescence *intensity*, F, and the lower curve, the fluorescence *yield*, F/I as function of the intensity of illumination, I). The second curve illustrates the fact, mentioned earlier in this

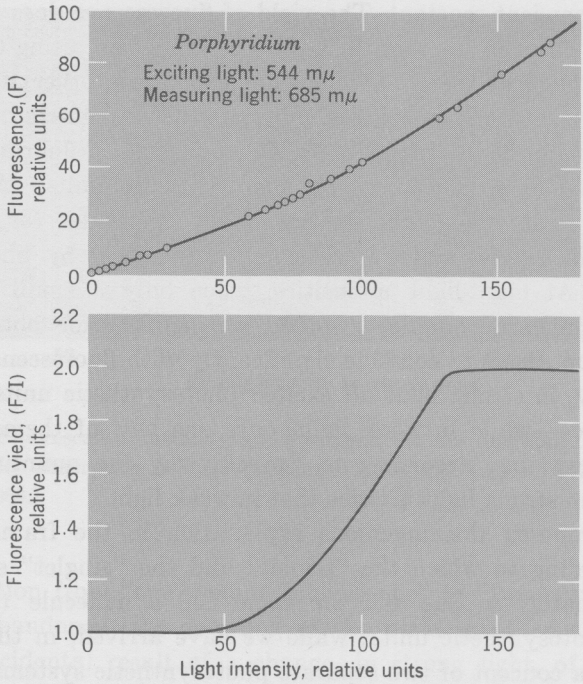

FIG. 15.9 Fluorescence intensity (F) and fluorescence yield (F/I) of *Porphyridium* as function of intensity of illumination. (A. Krey and Govindjee, 1964.)

chapter, that in light strong enough to saturate photosynthesis, the yield of fluorescence approximately doubles (rising from about 0.03 to about 0.06).

James Franck offered an interesting explanation for the doubling of the fluorescence yield at high light intensities. He suggested that, in photosynthesis, two primary photoreactions are brought about by chlorophyll molecules (or reaction centers in a photosynthetic unit), acting in the short-lived, fluorescent *singlet* state, and in the long-lived *metastable,* nonfluorescent *triplet* state, respectively. In the first case, photochemical action competes with fluorescence; in the second case, there is no such competition, because fluorescence is emitted before the molecules are transferred into the triplet state. The reason for this difference can be that the first reaction occurs practically instantaneously inside the "pigment complex," while the second requires a kinetic encounter

with an independent reactant. The yield of fluorescence does not depend, in the second case, on whether the triplet molecules are using their energy for a photochemical reaction, or losing it in some other way. During efficient photosynthesis, an equal number of reaction centers must operate in the singlet and in the triplet state. In low light, all fluorescence originates in the Chl *a* molecules (or photosynthetic units) that operate in the triplet state; while the fluorescence of molecules (or units) that operate in the singlet state, is completely quenched by photochemical competition. At high light intensities, when only a small fraction of the total number of quanta absorbed are utilized for photosynthesis, this utilization ceases to compete significantly with fluorescence emission. Consequently, in strong light *all* excited photosynthetic units contribute to fluorescence—while in weak light, only one half of them participate in it. This explains, according to Franck, why the quantum yield of fluorescence in strong light is twice that in weak light.

Franck proposed this ingenious explanation in the framework of a theory according to which the "triplet" and the "singlet" sensitization occurs alternately in one and the same Chl *a* molecule (or one and the same photosynthetic unit), while we have arrived, in the preceding chapters, at a concept of two separate photosynthetic systems sensitizing two successive primary photochemical processes. One could suggest that Franck's concept should be transferred to this picture, by assuming that one of the two systems operates in the singlet and the other in the triplet state; but closer analysis of the spectral composition of fluorescence in weak and in strong light do not support this hypothesis. Spectral analysis suggests that PSI contributes a weak but constant (intensity-independent) amount of fluorescence (F720) while PSII, which contributes most of the fluorescence in weak light (mainly F685) doubles its yield in strong light. In the red alga *Porphyridium cruentum*, a doubling of the quantum yield of fluorescence in strong light is observed when light is absorbed in the red pigment phycoerythrin (that belongs mainly to PSII); while no such effect is observed when excitation takes place in chlorophyll *a*, that is, predominantly in PSI (Fig. 15.10). The same effect as increased light intensity is caused by poisoning PSII selectively with poisons such as DCMU, [3- (3′, 4′ dichlorophenyl) 1,1-dimethyl urea]. Here, too, the weak PSI fluorescence remains unaffected, while the strong PSII fluorescence doubles or even trebles in intensity.

If Franck's explanation of the rise of Φ in strong light as resulting

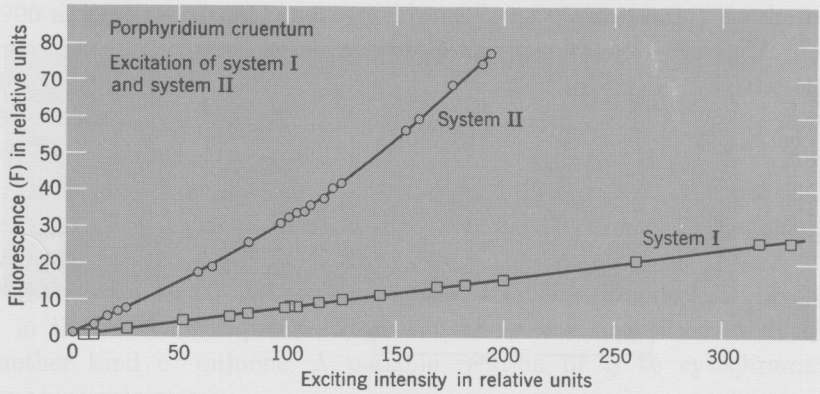

FIG. 15.10 Fluorescence intensity of *Porphyridium* as function of intensity of illumination for absorption in PSII (system II) and PSI (system I). (A. Krey and Govindjee, 1966.)

from transition from "one system fluorescence" to "two systems fluorescence" is abandoned, the doubling of the yield must be considered as a rather accidental result of a change, in strong light, of the relative rates of fluorescence emission and fluorescence quenching processes within system II.

THE TWO-LIGHT EFFECT

In 1960, we observed that chlorophyll *a* fluorescence in *Chlorella*, excited with red or blue light (absorbed in both PSI and PSII) became weaker when far-red light (absorbed mainly in PSI) was added. At the Agricultural Experiment Station at Beltsville, Md., Warren Butler found, in 1962, that preillumination with a strong orange-red light ("system II light"), causes, in *Chlorella*, enhanced fluorescence, while preillumination with strong far-red light ("system I light") weakened it. In Leiden (Holland), Duysens and Sweers (1963) superimposed a weak modulated light beam on a strong constant background light, and measured the fluorescence caused by the modulated beam. The "two-light effect" could be easily confirmed in this way (Fig. 15.11).

H. Kautsky and co-workers (1960) suggested that their fluorescence

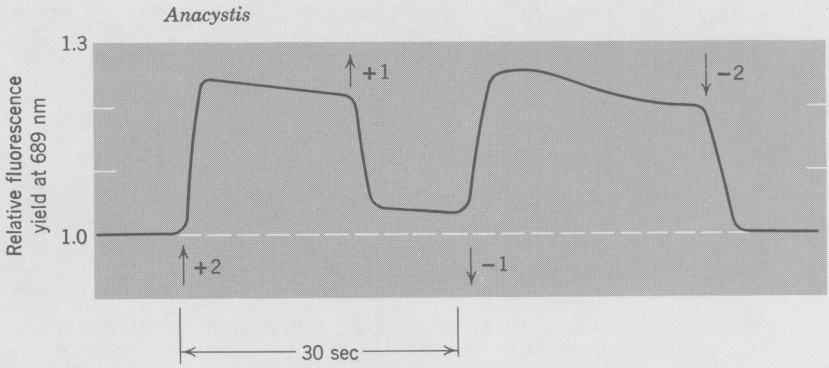

FIG. 15.11 Two-light effect on fluorescence of *Anacystis;* +2, addition of system II light; and +1, addition of system I light. (L. N. M. Duysens and H. E. Sweers, 1963.)

induction data could be best explained by postulating two light reactions in photosynthesis. Duysens proposed that, in PSII, electrons taken from water reduce an intermediate catalyst which he called Q. (The symbol Q stands for "quencher" of fluorescence.) The increase in fluorescence yield, caused by strong light absorbed in pigment system II, is due, he suggested, to accumulation of reduced (Q^-), which cannot accept electrons and is therefore a "nonquencher." When light absorbed in PSI is added, Q^- is reoxidized to Q, and fluorescence decreases.

Detailed studies of changes in PSII have been carried out in several other laboratories (including those at the University of Illinois, Kautsky's in Germany, Bessel Kok's at Baltimore, Jean Lavorel's and Pierre Joliot's in France), by observing changes in fluorescence during the induction period. These experiments provide the closest approach, so far, to the inner sanctum of photosynthesis—that is, to events that occur upon illumination in the photosynthetic pigment complex itself; but their complexity makes it impossible to describe them in detail in this introductory text.

Interpretations, suggested by several groups of observers, were all based on Fig. 14.4 according to which light absorption in PSII causes the reduction of a "primary oxidant" (labeled Y in the figure; Duysens' Q), which spreads into the whole enzymatic sequence between PSII and PSI. Light absorption in PSI reoxidizes this sequence, probably via

P700 as first reductant, and cytochrome *f* (or plastocyanin) as the next one. Certain experiments suggest that this reaction produces a "pool" of oxidized intermediates that can survive for several seconds; the effect of PSI absorption on PSII can be, therefore, observed even after a considerable dark interval (see Chapter 13, Fig. 13.7).

The position of *Q* in Fig. 14.4 suggests that its redox potential should be close to 0 volt. In Chapter 14, we have identified two such compounds, cytochrome b_3 and plastoquinone (PQ). Poisoning experiments with DCMU convinced Duysens that *Q* is *not* plastoquinone, but precedes it in the reaction sequence. However, there are suggestions that *Q* is another kind of quinone. A possible relation of *Q* to cytochrome b_3 remains unclear.

DELAYED FLUORESCENCE

An interesting phenomenon was discovered in 1951 by Bernard Strehler and William Arnold at Oak Ridge. They found that plants and bacteria emit light quanta for quite some time (many seconds) after the exciting light had been removed. This "delayed fluorescence" has a spectrum approximately identical with that of true chlorophyll *a* fluorescence; it is thus due to delayed formation of excited Chl *a* molecules. Delayed emission (at a certain time) shows a two-light effect: light absorbed in PSI quenches emission caused by excitation of PSII. The intensity of delayed emission is very weak (it can be discovered only by means of a "quantum counter," responding to a few photons); but because of its long duration, the contribution of this emission to steady-state fluorescence may be not negligible. The molecular mechanism of delayed emission is not yet clear. (The same is generally true of "chemoluminescence"—emission of visible quanta during chemical reactions.)

The occurrence of delayed fluorescence has led Arnold to look at photosynthetic units as pseudo-crystalline bodies, in which electrons liberated by light absorption can get stuck in "traps" or irregularities of the lattice, to be released at some later time. To what extent this "solid state picture" of the photosynthetic system is significant, remains an arguable question. (It is related to the alternative: "exciton migration or electron migration" mentioned in Chapter 12.)