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Fluorescence Properties of Chlorophyll *b*- and Chlorophyll *c*-Containing Algae

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ABBREVIATIONS AND SYMBOLS

BChl	Bacteriochlorophyll
Chl	Chlorophyll
Cyt *f*	Cytochrome *f*
DCPIP	2,6-Dichlorophenolindophenol
DCMU	3-(3,4-Dichlorophenyl)-1,1-dimethylurea; diuron
F_λ	Emission band at wavelength λ, in nanometers (nm)
FCCP	Fluorocarbonyl cyanide phenylhydrazone
I	Primary electron acceptor of PSII, pheophytin
kD	kilodalton
LDS	Lithium dodecyl sulfate

Light Emission by Plants and Bacteria

LHC	Light-harvesting complex
LHCP	Light-harvesting Chl *a*/Chl *b* protein complex
M_r	Molecular weight
O → I → D → P → S → M → T transient	Sequence of fluorescence levels during continuous illumination with time (O, origin; I, inflection; D, dip; P, peak; S, semi-steady state; M, maximum; T, terminal steady state)
P680	Primary electron donor of PSII; reaction center Chl of PSII
P700	Primary electron donor of PSI; reaction center Chl of PSI
PQ	Plastoquinone
PSI	Photosystem I
PSII	Photosystem II
SDS–PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

ABSTRACT

Light energy absorbed by photosynthetic pigments is used in photochemistry or lost as heat (internal conversion) or light [fluorescence; phosphorescence; delayed light emission (DLE)]. In this chapter we shall provide a brief review on the fluorescence properties of greenish [chlorophyll (Chl) *b*-containing] and brownish (Chl *c*-containing) algae and their possible relevance to photosynthesis. In Section I we mention the origin and phylogeny of photosynthesizing organisms, including the prokaryotes, whose fluorescence is discussed in the accompanying chapters. In Section II we describe the light-harvesting, i.e., the antenna, system; this includes a description of the photosynthetic pigments (Chl *a*, Chl c_1, Chl c_2, fucoxanthin, peridinin, etc.) and the pigment–protein complexes [light-harvesting complex (LHC), Chl *a*–Chl *c*–carotenoid complexes, etc.]. Except for some of the peridinin–Chl *a* complexes, the pigment–protein complexes are intrinsic proteins embedded in the thylakoid membrane. The transfer of excitation energy from Chl *b* and Chl *c* to Chl *a* is highly efficient, but that from the various carotenoids to Chl *a* is of variable efficiency. In Section III the significance of the various measured fluorescence parameters for photosynthesis is discussed. These discussions include: (a) the lifetime, τ, of fluorescence, which provides information on the excited states involved and relates the quantum yield of fluorescence, ϕ_f, to the natural lifetime of fluorescence, τ_0, which in turn is related to light absorption; (b) the quantum yield of fluorescence, which is a measure of the probability of light emission with respect to the probabilities of all other deactivation pathways of the excited state; (c) emission spectra, which provide information on the composition and the character of the emitting species; (d) the excitation spectrum of fluorescence, which provides information on the composition of the pigment systems and on the efficiency of the excitation energy transfer; (e) fluorescence induction under continuous illumination (also called fluorescence transients or the Kautsky phenomenon), which provides information on the electron flow on the electron donor and acceptor side of photosystem II (PSII) and its interaction with PSI; (f) flash-induced changes, which provide information on the primary photochemistry and the subsequent reactions in PSII, such as the electron flow that restores P680 from $P680^+$, where P680 is the reaction center Chl *a* of PSII, and the electron flow that leads to the recovery of Q_A from Q_A^-, where Q_A is the first quinone electron acceptor of PSII; and (g) fluorescence polarization, which provides information on the excitation energy migration and on the orientation of pigments *in vivo*. Recent studies cited in Section IV show that fluorescence studies in algae can also provide information on the regulation of excitation energy distribution and redistribution between the two photosystems or photosynthesis.

I. Introduction

This chapter deals with the fluorescence properties of Chl *b*- and Chl *c*-containing greenish (Chlorophyta, Euglenophyta) and brownish (Chromophyta, Dinophyta) algae. Mention will also be made of the prokaryotic greenish chloroxybacterium *Prochloron,* since it contains Chl *b*. Fluorescence characteristics of the phycobilin-containing algae (Rhodophyta, Cyanophyta (or Cyanobacteria), and Cryptophyta) and of *Prochloron* are discussed by Fork and Mohanty in Chapter 16, of the photosynthetic bacteria (both purple and green) by Amesz and Vasmel in Chapter 15, and of the primitive halobacteria by R. Govindjee and Ebrey in Chapter 14 of this volume. The bioluminescence properties of dinoflagellates (Dinophyta) are discussed by Hastings in Chapter 13.

This book deals with the *fluorescence* of various organisms, but we shall digress somewhat and discuss the *evolution* of some of these organisms. It is now generally believed that eukaryotic algae have evolved, in several parallel lines, by symbiosis of prokaryotic photosynthesizing bacteria with other bacteria or protozoa (see, e.g., Margulis, 1981). The details of the process are unknown, and the origin and phylogeny of eukaryotic algae are highly controversial. However, it is not too far-fetched to consider that the prokaryotic *Prochloron* (Chloroxybacterium), which is closely related to cyanobacteria, may be an ancestor of the green algae (Chlorophyta) and/or the euglenids (Euglenophyta) in view of the presence of Chl *b* in all these three groups. In the same way, one could speculate that cyanobacteria may be an ancestor of red algae (Rhodophyta) since they both contain phycobilins and phycobilisomes. Cryptomonads (Cryptophyta) are an interesting group since they contain phycobilins and Chl *c* but no phycobilisomes; their origin is uncertain. We speculate that all the Chl *c*-containing brownish algae (Chromophyta) may have evolved from as yet undiscovered Chl *c*-containing bacteria. [The reader should note that Chromophyta include the brown algae (Chrysophyceae), yellow-green algae (Xanthophyceae), and diatoms (Bacillariophyceae), and contain the carotenoid fucoxanthin.] On the other hand, many of the Chl *c*-containing dinoflagellates (Dinophyta), which contain the carotenoid peridinin instead of fucoxanthin, must have evolved separately as they are quite distinct from the

Chromophyta in their morphology. Wilcox and Wedemayer (1985) suggested that the "blue-green" chloroplast of the dinoflagellate *Amphidinium wigrense* has evolved from an endosymbiotic eukaryote (a cryptomonad).

In contrast to algae, the origin and phylogeny of prokaryotes are somewhat better known (see Fig. 1, based on discussions with C. Woese and D. Blubaugh) from the nucleotide sequences of their RNAs (ribonucleic acids) (Fox *et al.*, 1980). There are 10 (almost parallel) lines of evolution from a "common ancestor." We speculate the existence of an eleventh Chl *c*-containing line (see dashed line, Fig. 1). Out of the 10 lines, there are 5 photosynthesizing lines: (1) *Heliobacterium chlorum* (a gram-positive, BChl *g*-containing bacterium); (2) chloroxybacteria (e.g., *Prochloron*) and cyanobacteria (the only Chl *a*-containing O_2-evolving line); (3) purple bacteria (BChl *a*- or BChl *b*-containing), which are divided into three subgroups: the α and β groups (both are purple nonsul-

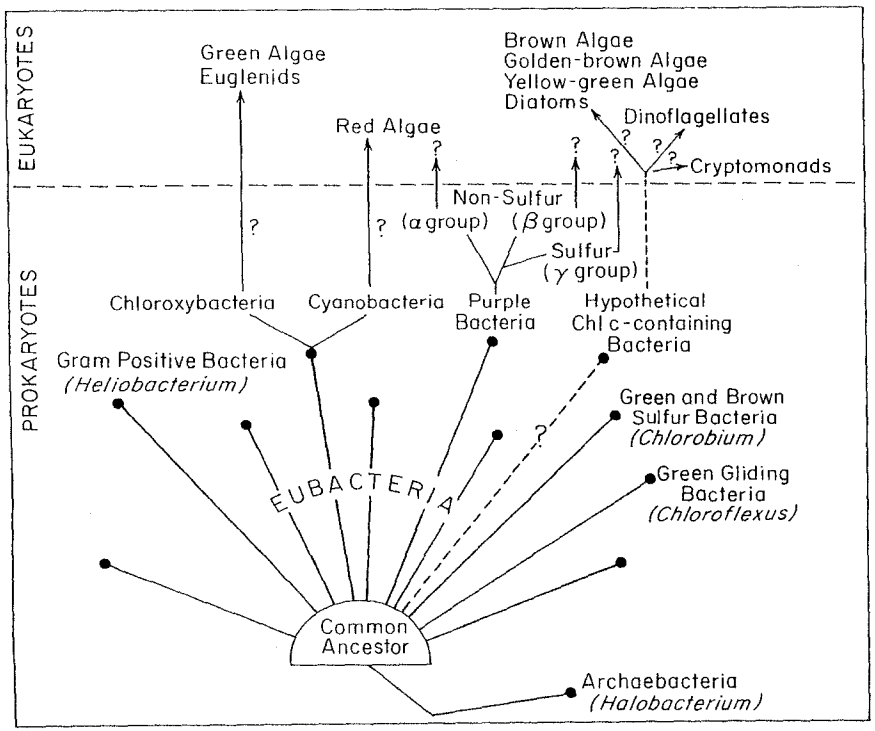

FIG. 1. Working hypothesis for the evolution of algae, cyanobacteria, and photosynthetic bacteria. See text.

fur bacteria, Rhodospirillaceae) and the γ group (purple sulfur bacteria, Chromatiaceae) (see Woese *et al.*, 1984a,b); (4) green and brown sulfur bacteria (Chlorobiaceae), which, in addition to BChl *a*, contain BChl *c* or *d* or *e*; like PSI of green plants, they possess FeS centers as secondary electron acceptors; and (5) green gliding bacteria (e.g., *Chloroflexus*), which are like the green sulfur bacteria in their pigment content but resemble the purple bacteria in their photochemical reactions (see Amesz, 1983; Blankenship, 1984, 1985). All the bacteria mentioned thus far are evolutionarily as far removed from the eukaryotes as they are from the so-called archaebacteria, which contain, among others, methanogens, and the light-transducing, bacteriorhodopsin-containing, H^+-pumping, and ATP-synthesizing *Halobacterium halobium*.

In the following, we shall first discuss the light-harvesting systems and then fluorescence from greenish (Chl *b*-containing) and brownish (Chl *c*-containing) algae. However, Chl *b*-containing *Prochloron* and Chl *c*-containing cryptomonads are discussed more fully by Fork and Mohanty (Chapter 16, this volume). For earlier reviews on Chl *a* fluorescence, see Govindjee and Papageorgiou (1971), Govindjee and Mohanty (1972), Goedheer (1972), Govindjee *et al.* (1973), Govindjee and Braun (Zilinskas) (1974), Mohanty and Govindjee (1974), Papageorgiou (1975), Lavorel and Etienne (1977), and Krause and Weis (1984). For a background on general aspects of photosynthesis, the reader is referred to Clayton (1980) and earlier volumes edited by Govindjee (1975, 1982a,b).

II. Light-Harvesting Systems

A. General

In photosynthesis of all plants and photosynthesizing bacteria, a collection of pigment molecules act as an antenna in absorbing light energy and transferring the excitation energy to specialized reaction center Chl *a* (or BChl) molecules, where the primary photochemistry occurs. This assemblage of pigments is referred to as the *photosynthetic unit*, and it contains the light-harvesting system of photosynthesis. It is now generally accepted that there are two light-harvesting pigment systems (PSI and PSII; Duysens *et al.*, 1961), with PSI being weakly fluorescent and PSII being somewhat more strongly fluorescent (see, e.g., Lavorel and Etienne, 1977). The electron transport pathway and the electron carriers involved in PSI and PSII reactions are shown in Fig. 1 in Duysens, Chapter 1, and Sane and Rutherford, Chapter 12, in this volume.

Before we begin a discussion of the light-harvesting system, we shall first review some well-known aspects of Chl *a* fluorescence in order to be

able to discuss the data in a rational fashion in this chapter. (For original references and a detailed description of this phenomenon, see Chapter 1 by Duysens and Chapter 10 by van Gorkom in this volume.) Fluorescence originating during the energy transfer process, i.e., before the photochemistry occurs, is labeled O level fluorescence (or, simply F_0); PSI contributes very little to F_0. As photochemistry occurs in PSII, and the quinone electron acceptor Q_A is reduced, the fluorescence yield of PSII increases. This fluorescence is known as the "variable" fluorescence, and is suggested (see Klimov *et al.*, 1978) to originate as

$$P680 \cdot I \cdot Q_A^- + h\nu \xrightarrow{(1)} P680^* \cdot I \cdot Q_A^- \xrightarrow{(2)} P680^+ \cdot I^- Q_A^-$$
$$\xrightarrow{(3)} P680^* \cdot I \cdot Q_A^- \xrightarrow{(4)} P680 \cdot I \cdot Q_A^- + h\nu'$$

where P680 is the reaction center Chl *a* of PSII and I is the primary electron acceptor pheophytin. The exciton created in reaction (3) is transferred back to the antenna molecules, and it appears from there as fluorescence, more appropriately as delayed fluorescence since the exciton was created by charge recombination in reaction (3) above (cf. van Gorkom, Chapter 10, and Jursinic, Chapter 11, this volume, for a critical discussion of this point). (Also see Note Added in Proof.)

Mauzerall (1985) recently succeeded in observing a lag or a rise time of 150–200 ps in the variable Chl *a* fluorescence yield from the green alga *Chlorella*. This finite rise time demonstrates an intermediate step between excitation and emission of the variable yield. One of the simplest interpretations is that 150–200 ps is the time for reaction (2) above, and that step (3), i.e., charge recombination, produces the variable fluorescence. The emission yield is low under the conditions $P680^+Q_A^-$, $P680 \cdot Q_A$, and $P680^+Q_A$, and is high only when both P680 and Q_A are in the reduced state: $P680 \cdot Q_A^-$.

The following is a description of the light-harvesting system in several Chl *b*- and Chl *c*-containing algae. We shall first describe the pigments, and then the pigment–protein complexes.

B. Photosynthetic Pigments

Chl *a* is present in all O_2-evolving photosynthetic organisms in the antenna as well as in the reaction center complexes (see, e.g., Prézelin and Alberte, 1978; Satoh and Butler, 1978; Anderson and Barrett, 1979; Green *et al.*, 1982). Chl *b* and Chl *c* serve only as light-harvesting pigments. Chl *b* is different from Chl *a* in only one group (see Fig. 1 in Chapter 5 by Seely and Connolly, this volume): CH_3 on ring II is replaced by CHO. Chl *c* is different from Chl *a* in that (1) it does not

contain the phytol side chain, (2) it has an unsaturated ring IV, and (3) it has an acrylic acid instead of a propionic acid side chain and a phytol on ring IV. These differences make Chl *c* more polar than both Chl *a* and Chl *b*. Compared to Chl *a* and Chl *b*, the absorption spectra of the two types of Chl *c* (c_1 and c_2; see, e.g., Jeffrey, 1969) are characterized by (1) weaker absorption in the red region relative to that in the blue region, and (2) a shift of the red band to shorter wavelengths and of the blue band to longer wavelengths (Fig. 2).

Chl *b* is found in green algae (Chlorophyta), in Euglenophyta, and in the green prokaryote *Prochloron*, whereas Chl *c* of two types, c_1 and c_2, is

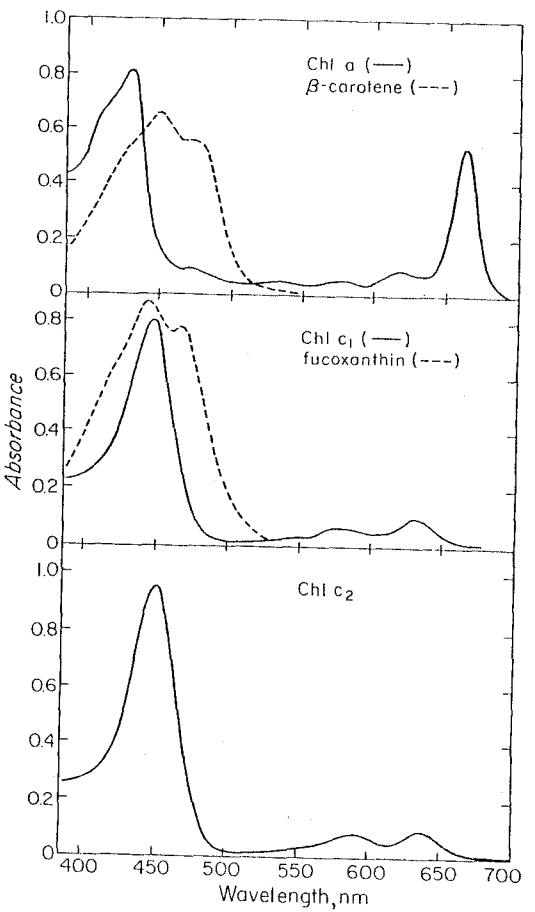

FIG. 2. Absorption spectra of several photosynthetic pigments *in vitro*. Spectra for Chl c_1 and Chl c_2 were redrawn from Jeffrey (1969).

TABLE I
Major Photosynthetic Pigments in Green and Brown Algae, Euglenids, Diatoms, and Dinoflagellates

Source	Chlorophylls	Main carotenoids
Dinoflagellates	a, c_2	Peridinin
	a, c_1, c_2	Fucoxanthin
Diatoms	a, c_1, c_2	Fucoxanthin
Brown algae	a, c_1, c_2	Fucoxanthin
Green algae	a, b	β-Carotene
Euglenophyta	a, b	β-Carotene

widely distributed in various marine brownish algae, diatoms, brown aglae, and dinoflagellates (Table I). Most cryptomonads (Cryptophyta) have only Chl c_2 (Jeffrey, 1969, 1976; see Fork and Mohanty, Chapter 16, this volume). Dinoflagellates (Dinophyta) that have the carotenoid fucoxanthin contain both Chl c_1 and c_2, but most of those that have the carotenoid peridinin possess only Chl c_2 (Jeffrey, 1976).

Like the chlorophylls, carotenoids [e.g., fucoxanthin (also called fucoxanthol) and peridinin] have an important role in collecting light energy (Tanada, 1951; Duysens, 1952; Haxo, 1960; Goedheer, 1970). The absorption peaks of fucoxanthin and peridinin in cells are usually shifted to about 20 nm to longer wavelengths compared to those of the same pigments in organic solvents (Fig. 2).

C. *Pigment–Protein Complexes*

It is generally believed that almost all photosynthetic pigments are associated with proteins; i.e., they exist as pigment–protein complexes. In plants there are essentially six light-harvesting complexes: two reaction center (RC) Chl *a* complexes (RC I or RC II complex) containing the reaction center Chl *a* (P700 or P680) and possessing several molecules of Chl *a* serving as the antenna; another set of two Chl *a* complexes (core Chl *a* complexes, CC-I or CC-II) containing only antenna molecules; and finally, two light-harvesting Chl *a*/Chl *b* protein complexes (LHCP-I or LHCP-II), again containing only antenna molecules. All six complexes act as the light-harvesting system of photosynthesis. The major difference between PSI and PSII is that the LHCP-II contains much more Chl *b* than the LHCP-I, and the Chl *a*-containing antenna of PSI possesses additional long-wavelength absorbing forms of Chl *a*.

The reaction center complexes of PSI and PSII have been isolated from various algae; they all contain *only* Chl *a*. In the following we shall

mainly discuss the peridinin–Chl *a*, Chl *a*/Chl *c*, and Chl *a*/Chl *b* complexes. For an excellent background on the photosynthetic pigments and models for their organization *in vivo*, the reader is referred to a review by Thornber and Barber (1979).

1. PERIDININ–CHLOROPHYLL *a* PROTEINS

Although it had already been known in the nineteenth century that at least part of peridinin is bound to a protein *in vivo* in dinoflagellates (Shütt, 1890),the nature of this pigment–protein was not studied until Haidack *et al.* (1966) isolated a water-soluble peridinin–Chl *a* protein from *Gonyaulax polyedra*. Further studies (Prézelin and Haxo, 1976) showed that it is composed of a single polypeptide of 32 kD and that it contains four peridinins and one Chl *a* per protein. By contrast, *Glenodinium* sp. has two peridinin–Chl *a* proteins which are similar in M_r (about 35,000) but different in isoelectric point (pI, 7.4 and 7.3, respectively). For further details, see Siegelman *et al.* (1977).

The excitation spectrum of Chl *a* fluorescence in the chromoprotein from *Amphidinium carterae* (Fig. 3) suggests that light energy absorbed by peridinin is transferred efficiently to Chl *a* (Haxo *et al.*, 1976). Song *et al.* (1976) and Koka and Song (1977) have shown that each Chl *a* is surrounded by two pairs of peridinin molecules with an appropriate orientation to ensure efficient energy transfer from peridinin to Chl *a*. These peridinin–Chl *a* proteins are the light-harvesting pigment–proteins and

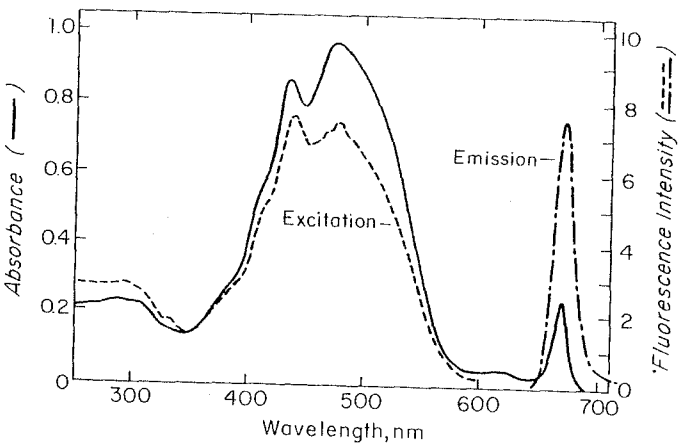

FIG. 3. Absorption, action, and emission spectra of fluorescence of a peridinin–chlorophyll *a*-protein complex from *Amphidinium carterae*. Solid line, absorbance; dashed line, action spectrum; and dash-dotted line, emission spectrum. Redrawn from Haxo *et al.* (1976).

are, perhaps, located peripherally on the photosynthetic membrane (Prézelin and Alberte, 1978) since they are easily extracted from the system (Haidack *et al.*, 1966; Prézelin and Haxo, 1976). These pigment–protein complexes are highly variable in amount, depending on the culture conditions (Prézelin, 1976; Prézelin and Haxo, 1976). In addition to the water-soluble proteins discussed above, there are also intrinsic membrane pigment–proteins which contain peridinin and Chl *a* (see, e.g., Boczar *et al.*, 1980).

2. CHLOROPHYLL *a*/CHLOROPHYLL *c* PROTEINS

All Chl *c* seems to be bound to intrinsic proteins to form light-harvesting Chl *a*/Chl *c* proteins. A comparison of the absorption and fluorescence excitation spectra of Chl *a*/Chl *c* proteins suggests efficient energy transfer from Chl *c* to Chl *a* (Anderson and Barrett, 1979; Alberte *et al.*, 1981). Most of the Chl *a*/Chl *c* proteins also contain the carotenoids (peridinin or fucoxanthin), which also function as light-harvesting pigments. Like other light-harvesting pigment–proteins (Prézelin and

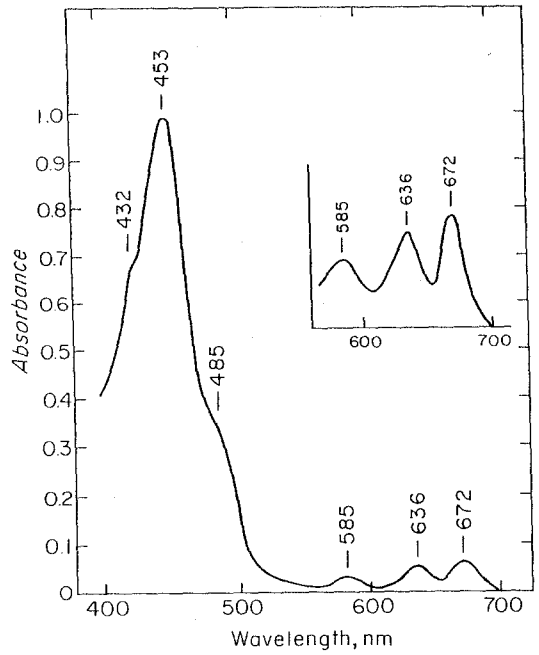

FIG. 4. Absorption spectrum of a chlorophyll *a*–chlorophyll *c*–protein complex from *Glenodinium* sp. Inset shows an enlarged portion of the absorbance bands in the red region of the spectrum. Redrawn from Boczar *et al.* (1980).

Haxo, 1976; Boardman *et al.*, 1978), the amounts of Chl *a*/Chl *c* proteins vary with the culture conditions (Anderson and Barrett, 1979).

Among the four Chl proteins obtained by Boczar *et al.* (1980) from the dinoflagellates *Gonyaulax polyedra* and *Glenodinium* sp., one was enriched in Chl *c*, having a Chl *c*/Chl *a* ratio of about 4.8, and contained xanthophylls (fucoxanthin, etc.). Figure 4 shows the absorption spectrum of this Chl *c*-enriched protein, where the 636-nm band is due to Chl *c* and the 672-nm band to Chl *a*. For a recent description of a Chl *a*/*c* protein containing fucoxanthin from the yellow-green alga *Synura petersenii*, see Wiedemann *et al.* (1983). A number of similar pigment–proteins have been isolated from brown algae and diatoms, and their properties vary considerably depending on the algal species and the isolation methods used. Table II summarizes the results on the various Chl *a*/Chl *c*–carotenoid proteins from four different algae (*Hormosira, Acrocarpia, Lami-*

TABLE II
Light-Harvesting Pigment–Proteins in Brown Algae and Diatoms

Species	Ref.	Methods	Pigment–proteins	Fluorescence maximum (nm)
Hormosira sp. (brown alga)	Kirk (1977)	0.05% Triton X-100, hydroxyapatite chromatography	Chl *a*, *c*, fucoxanthin, β-carotene–protein (5.7 : 1.1 : 3.0 : 0.6)[a]	—
			Chl *a*, *c*, fucoxanthin, violaxanthin, β-carotene–protein (6.4 : 0.7 : 3.0 : 1.2 : 0.8)	—
Acrocarpia paniculata (brown alga)	Barrett and Anderson (1977, 1980)	1% Triton X-100, sucrose density gradient	Chl *a*, c_2, fucoxanthin–protein (2 : 1 : 2)	680
			Green complex: Chl *a*, c_1, c_2, violaxanthin–protein (8 : 1 : 1 : 1)	683
			Orange complex: Chl *a*, c_2, fucoxanthin–protein (2 : 1 : 2)	683
Laminaria saccharinia (brown alga)	Alberte *et al.* (1981)	SDS–PAGE	Chl *a*, *c*–protein (2 : 1)	680
			Chl *a*, fucoxanthin–protein (1 : 4–6)	676
Phaeodactylum tricornutum (diatom)	Gugliemelli *et al.* (1981)	Broken cells treated with sodium lauryl sarcosinate	Chl *a*, *c*, fucoxanthin–protein (1 : 1 : 4)	690

[a] Numbers in the parentheses are the ratios of the component pigments.

naria, and *Phaeodactylum*). Examination of this table shows that it is difficult to draw a general picture.

3. CHLOROPHYLL *a*/CHLOROPHYLL *b* PROTEINS

Such proteins have been isolated from several greenish algae (Table III). What is important to note is that Chl *a*/Chl *b* proteins are present not only in PSII, but also in PSI (CPO in *Chlamydomonas* and LHC I in *Codium*).

Following Apel *et al.* (1975) and Apel (1977), who obtained only two Chl proteins from *Acetabularia,* Green and Camm (1981, 1982) and Green *et al.* (1982) resolved seven Chl-containing bands by using the detergent β-octyl glucoside as a solubilizing agent. Of these seven bands, four contained both Chl *a* and Chl *b* (see Table III).

Following the early work of Kan and Thornber (1976) on the light-harvesting Chl *a*/Chl *b* protein from *Chlamydomonas,* Delepelaire and Chua (1981) obtained several Chl *a*/Chl *b* proteins from photosystem II (CP-II-a, -b, -c, -d, and -e, which had several polypeptides of M_r between 25,000 and 33,000 in various ratios). Wollman and Bennoun (1982) found another Chl protein (CPO), which also had several polypeptides between 19 and 28 kD. This complex emits fluorescence at 705 nm at 77°K, and was proposed to be the antenna complex of PSI.

TABLE III
Chlorophyll *a/b* Protein Complexes in Several Algae

Genus	Ref.	Chl *a/b* proteins	Characteristics
Acetabularia	Green *et al.* (1982)	CP29 (a : b ratio, 3.4)	Internal antenna of PSII (apoprotein, 29 kD)
		CPII (a : b ratio, 1.0)	Apoprotein, 27 kD
		CPII*	Oligomeric form of CPII
		D	Oligomeric form of CP29
Chlamydomonas	Delepelaire and Chua (1981); Wollman and Bennoun (1982)	CP0	Part of antenna of PSI (27.5, 27, 25, 23, 19 kD)
		CPII-a,-b,-c,-d,-e	LHCPII (33, 30.5, 27.5, 27, 26.5, 25 kD)
Chlamydobotrys	Brandt *et al.* (1982)	LHCPb	LHCPII (several polypeptides)
Euglena	Brown (1980)	LHCP	LHCPII (several polypeptides)
Codium	Anderson (1983); Chu and Anderson (1985)	LHCPI	Oligomeric form of LHCP 3
		LHCP3	LHCPII (several polypeptides)
		LHCI	LHC of PSI

Light-harvesting Chl *a*/Chl *b* proteins have also been isolated from *Chlamydobotrys* (Brandt *et al.,* 1982), *Euglena* (Brown, 1980), *Codium* (Anderson, 1983; Chu and Anderson, 1985), *Chlorella fusca* (Wild and Urshel, 1980), and *Ulva mutabilis* (Huskovd *et al.,* 1982). In a first approximation, these complexes are similar to those from higher plants (see Briantais *et al.,* Chapter 18, this volume). However, Chl *a*/Chl *b* proteins and CPI from *Prochloron* (Hiller and Larkum, 1985) seem to be quite different from those in green algae and higher plants. Comparison of Chl proteins from a wide variety of algae is important not only from the evolutionary point of view, but also from the point of view of understanding the structure and function of Chl proteins.

III. Physical Parameters of Fluorescence

After a brief comparison of fluorescence of Chl *a* in solution and *in vivo,* we shall discuss some examples of fluorescence data obtained for the brownish (Chl *c*-containing) and the greenish (Chl *b*-containing) algae.

A comparison of the fluorescence characteristics of dilute solutions of Chl *a* and of Chl *a* in algae shows the following differences:

(a) The emission spectra are red-shifted by ~20 nm *in vivo* (685 nm) from that *in vitro* (665 nm) at room temperature; this emphasizes that Chl *a* in algae is in a distinctly different environment.

(b) The fluorescence yield (ϕ_f) (see Latimer *et al.,* 1956) and the measured lifetime of fluorescence (τ_f) are low (ϕ_f, 3–6%; τ_f, 1 ns, several components) compared to those *in vitro* (ϕ_f, 30%; τ_f, 5 ns), suggesting efficient energy utilization for photosynthesis in algae.

(c) Both the action and emission spectra of fluorescence in algae are complex and show the presence of several spectral forms of Chl *a in vivo;* under certain experimental conditions, especially at very low temperatures, the *in vivo* emission spectra of algae (e.g., *Chlorella*) may show up to six bands: F665 (a minor band from Chl *b*?), F680 (a minor band from Chl *a* of LHCP-II), F685 (Chl *a*-antenna of PSII, Chl *a*-II), F695 (reaction center complex II), F705 (an antenna of PSI), and F720 (another antenna of PSI).

(d) The degree of polarization of fluorescence in algae is low, and this may partly be due to extensive energy migration.

(e) Chl *a* fluorescence in algae undergoes characteristic changes with time of illumination (see, e.g., Govindjee and Papageorgiou, 1971; Papageorgiou, 1975; Briantais *et al.,* Chapter 18, this volume); such changes reflect the dynamic character of the photosynthetic system in algae; for

methods, see Lavorel *et al.* (Chapter 4, this volume) and Schreiber (1983).

A. Lifetimes and Yields

Lifetime of light emission provides information on the nature of the excited states involved. For example, if triplet states are involved, phosphorescence is observed which not only has a longer-wavelength emission than fluorescence, but also has a long lifetime (milliseconds) (see Hoff, Chapter 9, this volume). In photosynthesis, we usually deal with the singlet states (see Shipman, 1982), which last from picoseconds to nanoseconds (see Pearlstein, 1982). If the light emission is preceded by the creation of excitons by chemical back reactions (delayed fluorescence; see Jursinic, Chapter 11, this volume), its decay time reflects the time of the stabilization reactions and/or the actual recombination reactions. However, excitons created by light absorption decay by the prompt fluorescence pathway, basically, in competition with the excitation energy transfer, trapping at the reaction center, and other radiationless losses. The fluorescence decay and its lifetime can provide information not only on the reactions that cause it, but also on the heterogeneity of the pigment systems involved, their organization, and the excitation energy pathways and photochemical reactions of photosystems. We shall first briefly summarize the early work on the lifetime of fluorescence in algae, and then discuss our present understanding of this field, particularly in green algae. For references to early literature and for further details, the readers should consult Moya *et al.* (Chapter 7, this volume) and reviews by Lavorel and Etienne (1977), Govindjee and Jursinic (1979), and Karukstis and Sauer (1983).

The measured lifetime of fluorescence (τ_f) is related to the quantum yield of fluorescence (ϕ_f) by the simple relationship: $\tau_f = \phi_f \tau_0$, where τ_0 is the natural lifetime of the excited state, calculated from the integration of the absorption spectrum of the fluorescent state. Conclusions drawn in the early work (see, e.g., Brody and Rabinowitch, 1957; Müller and Lumry, 1965; Merkelo *et al.*, 1969; Müller *et al.*, 1969; Briantais *et al.*, 1972) on the green alga *Chlorella*, by both the flash and the phase methods, were: (1) some nonfluorescent (or weakly fluorescent) Chl *a* exists *in vivo*, (2) τ_f increases from a value of ~0.6 to ~2.0 ns as the reaction centers close, and (3) an exciton finding a reaction center closed migrates to pigment beds with open reaction centers. It was generally assumed that there is only one τ_f component. However, the existence of more than one τ_f component in photosynthetic systems, at room temperature, was demonstrated by Malkin *et al.* (1980) from their data on leaves of higher plants. What was measured in the older work was an average τ_f and new measurements with refined techniques now show several different values for τ_f, which can easily explain the older results (see Moya *et al.*, Chapter 7, this volume).

Haehnel *et al.* (1983) measured three lifetime components in the green algae *Chlorella vulgaris* and *Chlamydomonas reihardtii* by the single-photon direct flash method (Table IV). The interpretation of the three observed τ_f components (labeled τ_1, τ_2, and τ_3, Table IV) is not yet certain, but it is clear that τ_2 and τ_3 belong to PSII. The fastest component, τ_1, is in all likelihood made up of a PSI component and a PSII component (energy transfer from core Chl *a* II complex to RC II complex). On the basis of data with several mutants of *Chlamydomonas*, Gulotty *et al.* (1985) suggested that τ_1 of ~90 ps is composed of a 50-ps PSI and a 150-ps PSII component. Emission spectra for τ_1 in *Chlorella* by Kushida *et al.* (1981) and Wendler *et al.* (1984) support the PSI nature of τ_1. The τ_2 could reflect energy transfer either from LHCP-II to RC II or from one PSII to another independent PSII unit (called a "puddle" model). The τ_3 has been suggested to originate in charge recombination at the reaction center of PSII, with excitons created as $P680^+ \cdot I^- \rightarrow P680^*I$, where I refers to pheophytin; these excitons probably migrate to the antenna before being emitted (see Moya *et al.*, Chapter 7, this volume, and Karukstis and Sauer, 1983, for further discussion). Another explanation, which does not involve the charge recombination hypothesis, is that τ_3 simply originates in PSII α units where there is no barrier in energy transfer among different units (called a "lake" model) (see, e.g., Magde *et al.*, 1982, for interpretations concerning higher plant chloroplasts).

Further experiments on detergent-free PSI and PSII particles from algae are needed to answer questions regarding the identification of all the τ_f components.

TABLE IV
Lifetime of Fluorescence of Green Algae

Algae	Fluorescence level	τ_1	τ_2	τ_3	Ref.
Chlorella vulgaris	F_0	0.13	0.5	1.4	Haehnel *et al.* (1983)
	F_{max}	0.10	1.2	2.2	Haehnel *et al.* (1983)
Chylmydomonas reinhardtii	F_0	0.11	0.6	1.4	Haehnel *et al.* (1983)
	F_{max}	0.06	1.1	2.3	Haehnel *et al.* (1983)
	F_0	0.09	0.4	1.4	Gulotty *et al.* (1985)

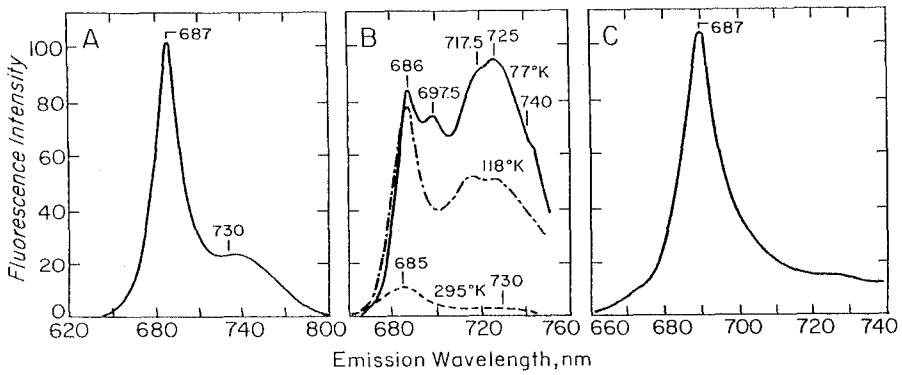

FIG. 5. Emission spectra of algae. (A) *Chlorella vulgaris* at room temperature (Ghosh *et al.*, 1966). (B) *Chlorella pyrenoidosa* at 77 and 118°K; for comparison, the room temperature spectrum is also shown (Cho and Govindjee, 1970a). (C) *Gonyaulax polyedra* (cultured in a light–dark cycle) at 77°K (Govindjee *et al.*, 1979).

At 77°K, τ_f is 2.3 and 1.4 ns, respectively, for F730 and F685 in *Chlorella* (Mar *et al.*, 1972; Hervo *et al.*, 1975). (See Moya *et al.*, Chapter 7, this volume, for further references and a detailed discussion of this topic.)

B. Emission and Excitation Spectra

1. EMISSION SPECTRA

a. Room Temperature: Multiplicity of Bands. In green algae, the room temperature fluorescence spectrum appears generally as a single major band at ~685 nm, accompanied by a vibrational band at ~740 nm (Fig. 5A; Duysens, 1952; Ghosh *et al.*, 1966). In brown algae and diatoms, the main emission band is often at 681 nm. In spite of the simplicity of this spectrum, it has been known for some time, based on a "matrix analysis"* (Weber, 1961) of fluorescence, that there is more than one emitting species even at room temperature in *Euglena* (Brody and Brody, 1963) and in *Chlorella* (Williams *et al.*, 1969). Recently, Marchiarallo and Ross (1985) extended this analysis to its ultimate form ("factor analysis") and provided emission spectra of the various emitting

* If fluorescence intensities at several wavelengths are measured after excitation with various wavelengths, the results can be set as an $m \times n$ matrix, with m columns determined by the wavelengths of excitation and n by the wavelengths of emission. The number of emitting components can be obtained from an analysis of this matrix (for details, see Weber, 1961).

components in several algae. More direct evidence for the existence of a band (or bands) around 700 nm in green algae was obtained from the following observations: (1) a band around 712 nm was inferred to exist when the emission spectrum at the maximum fluorescence (P level) was compared with that at the minimum fluorescence (O level) in *Chlorella*, the latter predominating in the 712-nm band (Lavorel, 1963), and (2) a small band around 695–700 nm was observed in the difference emission spectrum obtained when *Chlorella* cells were treated with different light regimes or with high and low light intensities (Papageorgiou and Govindjee, 1968b; Govindjee and Briantais, 1972).

Another small band at 665 nm was also noted in the difference spectrum, and was suggested to be due to Chl *b* emission (Govindjee and Briantais, 1972). To our knowledge, no emission from Chl *c* has been observed in intact cells of brown algae or diatoms since experiments similar to those on green algae have not yet been done with these organisms.

As surmised above, the green alga *Chlorella* has its main emission peak at ~685 nm in addition to at least one or more bands around 700 nm, excluding the vibrational band at 740 nm. In all likelihood, the 685-nm band is the strongly fluorescent PSII band and the 700-nm band is the weakly fluorescent PSI band. In other algae, however, distinct emission bands around 700–710 nm have been observed (for *Euglena* and *Ochromonas*, see Brown, 1966; Goedheer, 1981; for the green alga *Scenedesmus*, see Brown, 1967; Goedheer, 1981; for the diatom *Phaeodactylum*, see Brown 1967; Goedheer, 1973, 1981; and for the diatom *Detonula*, see Jupin, 1973). The band at 710 nm is often destroyed when the cells are broken (Brown, 1966). Several of these long-wavelength bands show fluorescence induction (see Section III,C), indicating that they may belong to PSII (Brown, 1967; Goedheer, 1973, 1981). However, a detailed analysis is not available to show that there was no significant contribution from the 685-nm band under the measuring conditions used and/or that the induction was not due to excitation energy transfer from PSII to PSI.

Mende *et al.* (1983) have shown that fluorescence spectra of *Chlorella* change during its life cycle, suggesting changes in energy distribution. It is important, therefore, that samples always be compared with cells of the same physiological age. Furthermore, stress effects can also be studied by measuring emission spectra [see, e.g., Harnischfeger and Jarry (1982) for changes in emission spectrum of *Chlorella* induced by cold treatment].

Chl *a* of PSI is only weakly fluorescent at room temperature. In addition to the emission bands mentioned above, some algae show distinct PSI fluorescence at ~720 nm (for *Chlorella vulgaris*, see Goedheer, 1981).

The PSI character of the 720-nm band at room temperature was confirmed by the fact that it did not show fluorescence induction and did not increase in intensity on addition of DCMU (Goedheer, 1981). DCMU is known to enhance PSII, not PSI, fluorescence by keeping Q_A in its reduced form Q_A^-, where Q_A is a quencher of fluorescence and Q_A^- is not (Duysens and Sweers, 1963).

b. Low Temperature. For low-temperature studies the reader is referred to reviews by Harnischfeger (1977) and by Amesz and Rijgersberg (1981). Brody (1958) discovered that, in addition to an emission band at 685 nm (F685), *Chlorella* cells have a new band at 725 nm (F720) at 77°K. Bergeron (1963), Brody and Brody (1963), Govindjee (1963), and Kok (1963) independently observed another new band at about 696 nm (F695) in the blue-green alga *Anacystis*, the green algae *Chlorella*, and *Scenedesmus*. It is now generally agreed that F685 and F695 belong to PSII and F720 to PSI. It was suggested that F695 originates in the reaction center complex of PSII (Govindjee, 1963); Breton (1983) suggested that F695 originates in pheophytin of the reaction center of PSII. Cho and Govindjee (1970a) showed that F720 is composed of at least two emission bands at 718 and 725 nm in *Chlorella* (see Fig. 5B), both of which originate in PSI. For studies on low-temperature spectra of mutants of *Chlorella sorokiniana*, the reader is referred to Lacambra *et al.* (1984).

In contrast to the broad band at 720 nm in most green and brown algae, this band in higher plants appears at 735 nm (see Briantais *et al.*, Chapter 18, this volume). Furthermore, in most cases the F735/F685 ratio is much higher in higher plant chloroplasts than the F720/F685 ratio in algae. This is often not due to a real difference; sometimes, it is due to higher reabsorption of short-wavelength components in either thick samples or samples having multiple path lengths (see Govindjee and Yang, 1966, for a relatively low F735/F685 ratio in a thin suspension of higher plant chloroplasts). Low-temperature fluorescence spectroscopy must be interpreted carefully because of the problems of artifacts. Several precautions must be taken (for a full discussion, see Govindjee, 1972) to reduce the possibility of reabsorption of the short-wavelength fluorescence bands: use of dilute samples; use of faster versus slower cooling (see, e.g., Fig. 1 in Cho and Govindjee, 1970a; Harnischfeger, 1977); use of front-surface optics; use of dimethyl sulfoxide as a protectant; and use of clear glass. Kramer (1984) has shown that excellent excitation and emission spectra can be obtained even with scattering samples. Kramer *et al.* (1985) have presented highly resolved 4°K emission and excitation spectra of *Chlorella vulgaris* and *Dunaliella salina*.

Furthermore, inclusion of a pigment that fluoresces at wavelengths different from that of the sample as a fluorescence standard is very useful in obtaining quantitative data at low temperatures.

Two fluorescence bands, F695 and F720, are almost absent in the dinoflagellates (Govindjee *et al.*, 1979) and in diatoms (Caron *et al.*, 1983) (Fig. 5C). We do not know whether the PSI pigment–protein complex in these algae is devoid of Chl *a* 695 responsible for F720, or whether there is another reason for the quenching of this band. The relative lack of F695 is more difficult to explain because it is predicted to arise from reaction center II. Further research is needed to understand the relationship of the four emission bands (F685, F695, F718, F725) to the various Chl *a* complexes in algae.

Cho *et al.* (1966) observed that as the temperature was lowered from 77 to 4°K, the F685/F695 ratio increased in *Chlorella* (Fig. 6). This suggested that the efficiency of excitation energy transfer from the core Chl

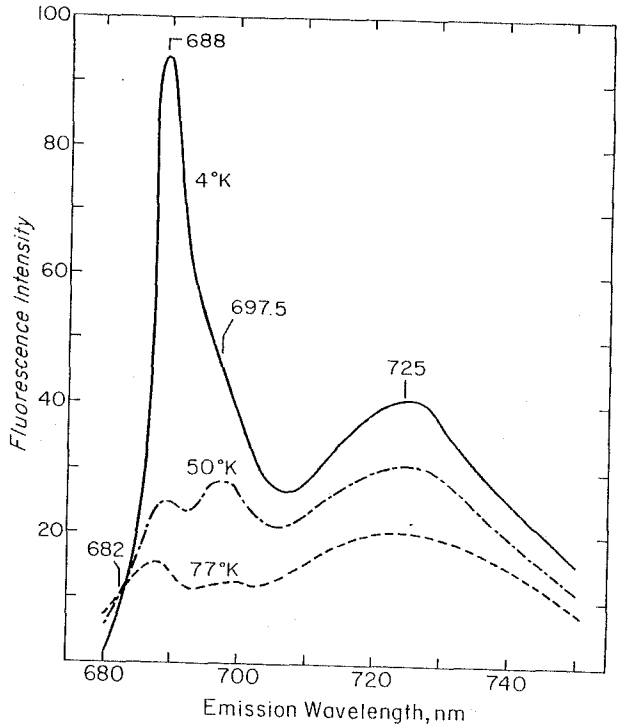

FIG. 6. Emission spectra of *Chlorella pyrenoidosa* at 4 and 50°K; for comparison, 77°K spectrum is also shown (Cho *et al.*, 1966).

a II complex to the reaction center II complex decreases at the lowered temperature. Experiments on the temperature dependence of emission spectra of *Chlorella* in the range 295 to 77°K revealed that the F720 band increased rather slowly up to 180°K, and then rapidly as the sample was cooled, whereas the F685 band increased rapidly up to 180°K, and then slowly (Cho and Govindjee, 1970a). One interpretation of these data is that the efficiency of energy transfer from PSII to PSI may decrease when the sample is cooled from 240 to 180°K, and then increase at still lower temperatures. Unfortunately, such conclusions are difficult to prove because changes in fluorescence intensity could arise for several other reasons as well. However, it is tempting to accept the notion of decreased energy transfer at lower temperatures due either to uncoupling of one Chl *a*–protein complex with the other, and/or to the reduction in the Förster overlap integral (see van Grondelle and Amesz, Chapter 8, this volume) between absorption and fluorescence due to sharpening of the bands at lower temperatures.

2. ACTION SPECTRA OF FLUORESCENCE

Action (excitation) spectra of fluorescence have been used extensively to study excitation energy transfer in photosynthesis of algae (see, e.g., the historical papers by Dutton *et al.*, 1943; Wassink and Kersten, 1946; Duysens, 1952). For an earlier review on action spectra, see Fork and Amesz (1969). In the well-known technique of sensitized fluorescence, excitation of a molecule B leads to fluorescence from another molecule A; this shows excitation energy transfer from B to A. The efficiency of this energy transfer can be calculated by measuring the action spectrum of the fluorescence of A, i.e., the fluorescence intensity of A per incident number of photons at different wavelengths of excitation, and the fractional absorbance spectrum of the system. The wavelength-dependent quantum yield spectrum of fluorescence of A, calculated from the above measurements, provides information on the efficiency of energy transfer. For example, if the quantum yield in the region of absorption of B is 50% of that in the region of absorption of A, then the efficiency of energy transfer is 50%. The earliest evidence for efficient (70–80%) excitation energy transfer in photosynthesis was from the carotenoid fucoxanthin to Chl *a* when fucoxanthin-sensitized Chl *a* fluorescence was observed in a diatom by Dutton *et al.* (1943). Goedheer (1970) reported a similarly high efficiency of excitation energy transfer, at 77°K, from fucoxanthin and from Chl *c* to Chl *a* in the diatom *Phaeodactylum tricornutum*.

a. Action Spectra at Room Temperature. The wavelength-dependent quantum yield spectrum of Chl *a* fluorescence at >690 nm in the green alga *Chlorella* shows a "red drop" around 685 nm suggesting the presence of nonfluorescent or weakly fluorescent Chl *a* molecules which preferentially absorb light beyond 685 nm (see, e.g., Szalay *et al.*, 1967; R. Govindjee *et al.*, 1968). The red drop in the fluorescence can be eliminated by aerobic sonication of *Chlorella* cells, and it can be related to the presence of a minor Chl *a* form absorbing around 695 nm (in all likelihood, a PSI component) which is weakly fluorescent at room temperature, but which is responsible for the fluorescence band at 720 nm (F720) at 77°K (Das and Govindjee, 1967). A consequence of the presence of a nonfluorescent or weakly fluorescent form of Chl *a*, in Chl *b*-containing algae, is that the ratio of Chl *b* to Chl *a* peaks is higher in the action spectrum of fluorescence than in the absorbance spectrum (see, e.g., Butler and Bishop, 1963; Goedheer, 1966).

A typical action spectrum of Chl *a* fluorescence at 740 nm at room temperature (mostly from PSII) shows peaks at about 440 nm (Chl *a*), 480 nm (Chl *b*), 650 nm (Chl *b*), and 670 nm (Chl *a*) in the green alga *Chlorella vulgaris* (see, e.g., Ghosh *et al.*, 1966; Fig. 7). A comparison of the action spectrum of fluorescence with the absorbance spectrum in

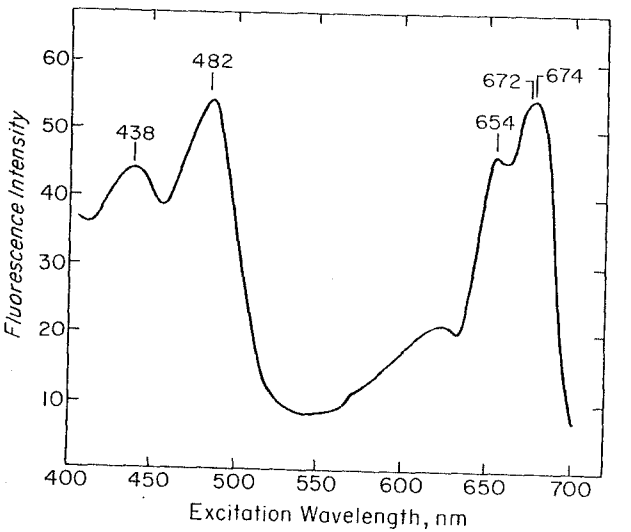

FIG. 7. Action spectrum of fluorescence at 740 nm at room temperature from *Chlorella vulgaris* (Ghosh *et al.*, 1966).

Chlorella reveals that the efficiency of energy transfer from Chl *b* to Chl *a* is 100%, but the efficiency of energy transfer from the various carotenoids (absorbing in the 400–540-nm region; see Govindjee, 1960) to Chl *a* is somewhat lower (~60%). For a discussion of efficient energy transfer from the carotenoid siphonoxanthin to Chl *a* in the seaweed *Ulva japonica*, see Kageyama *et al.* (1977).

b. Action Spectra at Low Temperatures. The action spectra of Chl *a* fluorescence in Chl *b*- or Chl *c*-containing cells at 77°K are narrower than those obtained at room temperature in each case due to the sharpening of the absorption bands.

In the dinoflagellate *Gonyaulax polyedra*, the action spectrum of Chl *a* fluorescence at 750 nm, in the presence of DCMU, and at 77°K (F_{max}, maximum fluorescence intensity) shows peaks at 672 nm (Chl *a*) and 636 nm (Chl *c*) and a broad but low band in the 400–560-nm region (due to carotenoids diadinoxanthin, dinoxanthin, and peridinin) (Govindjee *et al.*, 1979). The efficiency of energy transfer from Chl *c* to Chl *a* has been shown to be very high (~100%). Furthermore, the efficiency of energy transfer from peridinin to Chl *a* in isolated complexes has been shown to be very high. (However, there are many carotenoids *in vivo* that are not present in the photosynthetic membranes, but are in the cell walls, and are thus responsible for an apparently low calculated efficiency of energy transfer from carotenoids to Chl *a*.)

At low temperatures (77°K), the green alga *Chlorella* shows four emission bands: F685 (PSII), F695 (PSII), F718 (PSI), and F725 (PSI) (see, e.g., Fig. 5B; Cho and Govindjee, 1970a,b). Thus, action spectra for Chl *a* fluorescence can be measured for the various components in *Chlorella*. Figure 8A shows the absorbance spectrum at 4°K with peaks at 440 (Chl *a*), 464 (carotenoids), 477 (carotenoids + Chl *b*), 491 (carotenoids), 650 (Chl *b*), 670 (Chl *a*), and 678 nm (Chl *a*). Action spectra of fluorescence for F685, F695 (Fig. 8B), and F725 (Fig. 8C) measured at various temperatures from 77 to 4°K shows two important features: (1) the contribution of Chl *b* (peaks at ~480 and ~650 nm) with respect to Chl *a* (670, 678 nm) is greater for F685 and F695, suggesting that they are from PSII, in contrast to that for F725, and (2) the ratio of the Chl *b*/Chl *a* peaks is almost independent of temperature (Cho and Govindjee, 1970b). Furthermore, action spectra measured with narrow slit widths (~1 nm) for F685 (Fig. 8D) and F695 show that the pigments funneling energy to these components are almost identical and that both Chl *a* 670 and Chl *a* 678 belong to the complexes fluorescing at these two wavelengths. Action spectra measured at several long wavelengths show clearly that F725 is sensitized by pigments absorbing at longer wavelengths than the fluorescence at ~760 nm (which arises from the vibrational band of the short-wavelength Chl *a* forms).

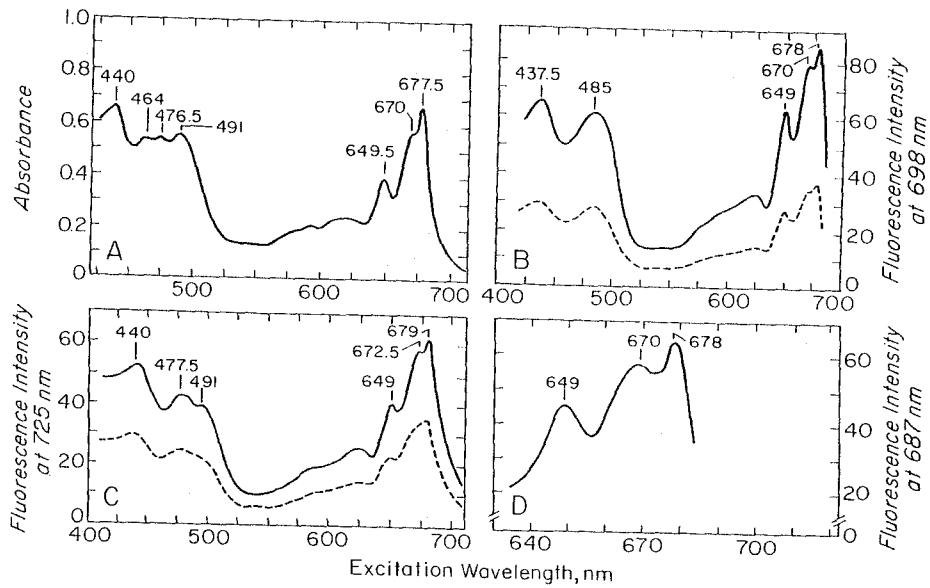

FIG. 8. Absorption spectrum and action spectra of fluorescence at 4–77°K from *Chlorella pyrenoidosa*. (A) Absorption spectrum at 4°K (spectrum at 77°K overlapped with that at 4°K). (B) Action spectra at 4 (solid line) and 77°K (dashed line) for fluorescence at 698 nm (F695). (C) Action spectra at 4 (solid line) and 77°K (dashed line) for fluorescence at 725 nm (F725). (D) Action spectrum at 4°K in the red region of the spectrum for fluorescence at 687 nm. (Redrawn from Cho and Govindjee, 1970b.)

C. Induction of Chlorophyll a Fluorescence

Among algae, the unicellular green species *Chlorella* has been most extensively used for the study of induction of Chl *a* fluorescence. An advantage of green algae is that they show a rather simple fluorescence induction (Kautsky transient—see, e.g., Kautsky *et al.*, 1960; Munday and Govindjee, 1969a,b). However, the transients become complicated because state I–state II transitions (see, e.g., Williams *et al.*, 1980; Satoh and Fork, 1983; Catt *et al.*, 1984; Section IV, this chapter), high-energy-state-induced fluorescence quenching (Mohanty and Govindjee, 1973a,b; Krause *et al.*, 1983), or transients related to carbon dioxide fixation (Walker *et al.*, 1983) may begin to overlap the late stage of the Kautsky transients. We will summarize here mainly information on the

early stage of the fluorescence transients in green algae (for earlier reviews, see Govindjee and Papageorgiou, 1971, Mohanty and Govindjee, 1974; Papageorgiou, 1975; Krause and Weis, 1984). For studies on brown algae, see Berkaloff and Duvall (1980) and Bruce *et al.* (1983), and for studies on leaves and chloroplasts from higher plants, see Briantais *et al.*, Chapter 18, this volume.

Characteristic transients (Fig. 9) of the fluorescence induction in green algae are referred to as OIDPSMT following the terminology discussed in Govindjee and Papageorgiou (1971), but they are referred to here as $OIDPS_1M_1S_2M_2T$, as modified by Yamagishi *et al.* (1978) to include three peaks; this is based partially on the alternative terminology of Bannister and Rice (1968). A brief description of the terminology follows. The O (for origin) level, the so-called constant fluorescence, is the instantaneous fluorescence level obtained within the opening time of the shutter used to start the illumination (<1 ms). The level I stands for an inflection or an intermediate peak or hump, whereas the OI phase is photochemical and dependent on exciting light intensity. The level D is used for the dip which follows I. The highest level is P, which stands for peak or plateau; the IDP rise occurs within ~1 s, when the intensity of light just saturates photosynthesis. The PS decay occurs within 5 to 10 s. The S stands for semi-steady state. The SM rise occurs within 0.5 min; the symbol M stands for a maximum. The MT decline occurs within 2 min. The symbol T stands for terminal steady state. Sometimes there are two maxima M_1 and M_2 and then S is referred to as S_1 and the lower fluorescence point between M_1 and M_2 as S_2 (see Fig. 9). We emphasize that it is important for the interpretation of the data that the O level not be confused with the I level or with the first measured point (F_i), which may

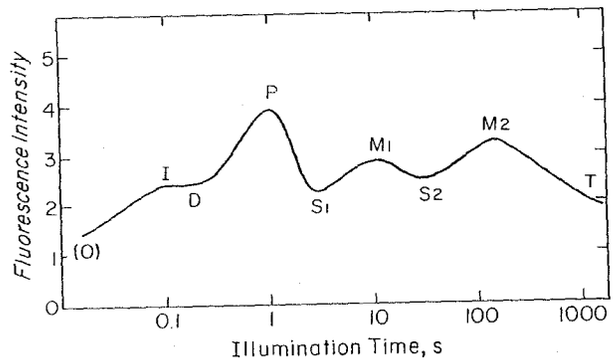

FIG. 9. Chlorophyll *a* fluorescence intensity as a function of time of illumination in *Bryopsis* cells. See text for definition of symbols. (Redrawn from Yamagishi *et al.*, 1978.)

lie anywhere between O and P depending on the opening time of the shutter and the exciting light intensity.

It is well established that the intensity of Chl *a* fluorescence *in vivo* is controlled by the redox state of Q_A, the first quinone electron acceptor of PSII; the fluorescence yield is high when Q_A is reduced and low when Q_A is oxidized (Duysens and Sweers, 1963). In addition, as noted above, the emission intensity is modulated by state transitions, the high-energy state of the thylakoid membranes, and cation transport across the membranes at physiological temperatures (see reviews by Papageorgiou, 1975; Lavorel and Etienne, 1977; Briantais *et al.*, Chapter 18, this volume).

1. THE O → I RISE

The initial fluorescence (O) is thought to be emitted from PSII during the time when excitation energy is transferred among antenna Chl *a* molecules and from the weakly fluorescent Chl *a* of PSI (Munday and Govindjee, 1969a) (see Table V). Thus, it represents the fluorescence of the system with open reaction centers, i.e., before photochemical trapping. Its intensity is linearly proportional to the incident light intensity, but is dependent on the distribution and redistribution of excitation energy between the two photosystems. Since this fluorescence originates as one of the deactivation pathways of antenna molecules, its level is also dependent on the connection between the antenna and the reaction

TABLE V
Explanation of Chlorophyll *a* Fluorescence Induction

Fluorescence levels and/or transients	Current interpretation
O level	Due to loss of excitation energy during transfer (PSII and PSI) to the reaction centers
OI rise	Reduction of Q_A to Q_A^- by PSII
ID decline	Reoxidation of Q_A^- to Q_A by PSI
DP rise	Accumulation of reducing equivalents in the electron transport chain up to PSI acceptors
PS decline	Complex—mostly explained by Q_A^- reoxidation. Current hypothesis includes role of O_2 (decline is absent under anaerobic conditions); see Fig. 10 and text
SM rise	Independent of redox state of Q_A; regulated by structural (state) changes. Alternatively, (1) ΔpH-dependent effect on electron flow; (2) regulation by Calvin cycle reactions
MT decline	Indirectly linked to ATP synthesis and utilization; structural (state) changes

center. In view of the fact that the energy trap of PSII, P680, and the antenna Chl *a* of PSII are of approximately the same energy, the trap is not irreversible, and the so-called constant fluorescence is not truly constant since the rate constants of various pathways at the reaction center would control this fluorescence yield. The OI rise and the ID plateau (or decline) are reflections of the initial rapid reduction of Q_A by PSII and the subsequent oxidation of reduced Q_A by PSI, respectively (Munday and Govindjee, 1969b; Satoh and Katoh, 1981). The I level increases with increasing intensity and is enhanced when the electron flow from Q_A^- to Q_B is blocked. Since there is a large plastoquinone (PQ) pool between PSI and Q_A, it takes several tens of milliseconds for PSI to oxidize Q_A^-. Thus the OI rise precedes the ID plateau or decline. In fact, the extent of the OI rise and the ID decline depend strongly on the redox state of the PQ pool (Jennings and Forti, 1975; Satoh and Katoh, 1981), and the ID decline is increased by PSI light (Munday and Govindjee, 1969a,b; Schreiber and Vidaver, 1974). The requirement of light for D is shown by the delay in its occurrence when it is measured with flashing light (with dark times between flashes) compared to that with continuous light. Furthermore, the ID decline is very pronounced under anaerobic conditions.

2. THE D → P RISE

It seems that during the D-to-P transition all electron carriers up to X (a PSI acceptor) are reduced, as suggested by the following observations: (1) intersystem electron carriers Cyt *f* (Satoh *et al.*, 1977) and P700 (Maxwell and Biggins, 1977) become fully reduced during the DP rise following the initial rapid oxidation during the OI rise, and (2) simultaneous measurements of O_2 evolution and Chl *a* fluorescence in *Chlorella* show that electron flow is greatly inhibited at the P stage (Delosme *et al.*, 1959; Joliot, 1965, 1968; Bannister and Rice, 1968; Govindjee and Papageorgious, 1971).

The classical explanation of the DP rise was that as the PSII reactions occur, the PQ pool is filled, which leads to accumulation of Q_A^-. This explanation assumes that the PQ pool is oxidized in dark-adapted samples, but is apparently contradicted by data suggesting that the PQ pool is in the reduced state in dark-adapted samples (see, e.g., discussion by Rutherford *et al.*, 1984, for intact leaves). Another possibility is that the electron transport on the acceptor (reducing) side of PSI is blocked in the dark-adapted state, most likely between ferredoxin and $NADP^+$. (The reader may consult Fig. 1 in Duysens, Chapter 1, or Sane and Rutherford, Chapter 12, this volume.) In this picture, the reduction of

Q_A apparently occurs subsequent to the reduction of intermediates between P700 and the blocked site. This idea seems to be consistent with the following observations. The specific PSI electron acceptors which remove electrons before ferredoxin (e.g., methyl viologen—Munday and Govindjee, 1969b; Lavergne, 1974; Satoh *et al.*, 1977; or nitrite—Kessler and Zumft, 1973; Satoh and Katoh, 1980) eliminate the DP transient in green algae. By contrast, oxidants that accept electrons after ferredoxin–$NADP^+$ reductase ($NADP^+$ in *Bryopsis*—Satoh, 1981; oxaloacetate, 3-phosphoglycerate in intact spinach chloroplasts—Satoh and Katoh, 1980) have little effect on the fluorescence transient. Furthermore, the DP rise seen in dark-adapted algal cells is decreased or abolished by preillumination (Duysens and Sweers, 1963; Mohanty and Govindjee, 1973a, 1974; Satoh *et al.*, 1977), possibly because the block mentioned above is removed during preillumination and thus Q_A^- does not accumulate and the P level is low.

For a quantitative analysis of fluorescence induction curves (O → I → P) in isolated chloroplasts from higher plants, see Renger and Schulze (1985); application to algae remains to be made.

3. THE P → S DECLINE

A complete explanation of the subsequent fluorescence decline from P to S is still more complex. For a long time it was thought that the P-to-S phase in *Chlorella* cannot be simply explained by the reoxidation of Q_A^- to Q_A even though the rate of O_2 evolution is complementary to fluorescence changes during this time (Lavorel, 1959; Duysens and Sweers, 1963; Mohanty and Govindjee, 1974). The P-to-S phase is a thermal phase since it can continue in darkness (Lavorel, 1959; Lavergne, 1974; Mohanty and Govindjee, 1974). Furthermore, it can be shown that the S level increases on preillumination with light I but decreases on preillumination with light II (Munday and Govindjee, 1969b; Mohanty and Govindjee, 1974), in contrast to the antagonistic effect of light I and light II on Chl *a* fluorescence yield, which is reduced by light I and increased by light II (Govindjee *et al.*, 1960; Butler, 1962; Duysens and Sweers, 1963; Mohanty *et al.*, 1970). The susceptibility of P-to-S decay to uncouplers of photophosphorylation, the absence of the decay under anaerobiosis, and its possible relationship to the redox state of the PSI acceptor side indicate that it is a complex phenomenon. Mohanty and Govindjee (1974) suggested that the PS decline in algae is due to an increase in the rate constant of internal conversion and/or energy transfer to weakly fluorescent Chl *a* molecules (state changes), which is a consequence of the structural changes in the membrane, which in turn are due to the

buildup of a "phosphorylation or ΔpH potential" because of increased noncyclic electron flow. Satoh *et al.* (1977) proposed a different working hypothesis to explain the PS decline (Fig. 10). They suggested that the block on the electron acceptor side of PSI (labeled "Dark-inactivated site" in the diagram) is first removed during the illumination (see section on D → P rise). Then, during the P-to-S phase, the reduced PSI acceptor is oxidized, which leads to subsequent reoxidation of Q_A^- to Q_A and thus lowered fluorescence at the S level. The observation that the activity of ferredoxin–$NADP^+$ reductase changes on transfer of algal cells from a dark to a light cycle (Satoh, 1981) suggests that this enzyme may be the site of light-dependent modulation of electron transport of the reducing (electron acceptor) side of PSI. The reactivation of PSI is also suggested by the observation that this P-to-S phase of the fluorescence induction is accompanied by reoxidation of Cyt *f* (see right side of Fig. 10). The PS decline is too rapid to be explained in terms of activation of the CO_2 fixation enzyme system. Furthermore, KCN, at concentrations where carbon fixation is completely inhibited, has no effect on the PS decline (Wassink and Katz, 1939; Satoh *et al.*, 1977).

The sensitivity of the PS decline to uncouplers of photophosphorylation (see Tables 1 and 2a in Mohanty and Govindjee, 1974) may be explained by the fact that uncouplers like CCCP (carbonyl cyanide *m*-chlorophenylhydrazone) decrease the internal pH of the chloroplasts, and the PS decline is highly sensitive to pH (see Briantais *et al.*, 1979, for isolated chloroplasts; Yamagishi *et al.*, 1981).

Oxygen seems to play an important role in the PS phase because the PS fluorescence decay is strongly suppressed under anaerobiosis

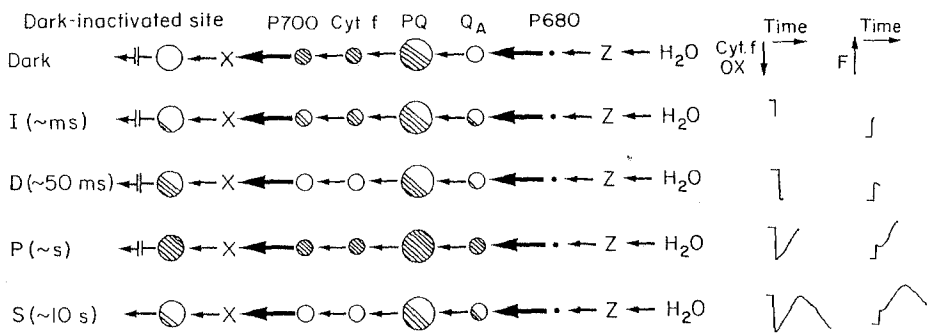

FIG. 10. Working hypothesis to explain the fluorescence transient. Z, electron donor to the reaction center chlorophyll *a* of photosystem II (P680); Q_A, electron acceptor of photosystem II; PQ, plastoquinone pool; cyt *f*, cytochrome *f*; P700, reaction center chlorophyll *a* of photosystem I. See text for details.

(Kautsky and Frank, 1943; Shiau and Franck, 1947; Bannister and Rice, 1968; Munday and Govindjee, 1969a; Papageorgiou, 1975; Satoh, 1982). However, preillumination lowers the P level when measured under aerobic conditions, but not under anaerobic conditions, indicating that even after the preillumination, electron flow through PSI is limited when O_2 is absent (Satoh, 1982). This suggests that O_2 serves as an electron acceptor (also see Radmer and Ollinger, 1980). Perhaps during this pseudocyclic electron flow the system is being prepared for the initiation of CO_2 fixation.

For the relationship of fluorescence transients to photoacoustic signal measurements in *Bryopsis*, see Katoh and Yamagishi (1984). Results reported in that paper are apparently consistent with the hypothesis for P-to-S decay shown in Fig. 10. Further research is necessary to fully understand the mechanism of P-to-S decay in algae and to relate it to similar, although quite distinct, changes in higher plants (see Briantais *et al.*, Chapter 18, this volume).

4. THE S → M RISE

The S to M portion of the fluorescence transient seems to be independent of the redox state of Q_A since the rate of O_2 evolution and the SM phase increase in parallel in *Chlorella* (Papageorgiou and Govindjee, 1968a). Slovacek and Bannister (1973) showed that in CO_2-depleted *Chlorella* cells the SM phase is suppressed, but the addition of NH_4Cl revives a large SM rise. Although the latter authors argued against the SM rise being related to the state changes, structural changes facilitating the transformation of the system to the highly fluorescent state I cannot be ignored (see Section IV). On the other hand, Walker *et al.* (1983), working with higher plants, found an antiparallel relationship of the SM rise with O_2 evolution (although out of phase) and related these changes to decreased NADPH reoxidation and increased utilization of ATP in the Calvin cycle.

In the alga *Bryopsis*, which shows two distinct peaks, M_1 and M_2, the fluorescence increase up to M_1 was shown to be related to the formation of ΔpH across the thylakoid membranes (Yamagishi *et al.*, 1978). Uncouplers that dissipate ΔpH suppress the SM_1 rise, whereas energy transfer inhibitors and valinomycin, which increase ΔpH, magnify the fluorescence rise. The transient was, therefore, ascribed to partial reduction of Q_A caused by control of electron transport by ΔpH. However, in *Chlorella* (1) Papageorgiou and Govindjee (1968a) showed that atabrin, but *not* FCCP and phloridzin, had a significant effect on the SM rise, (2) Papageorgiou and Govindjee (1971) showed that the SM rise in *Chlorella*

was unaffected by the pH of the culture medium, and (3) the formation of ΔpH is expected to decrease the electron flow, but O_2 evolution was found to increase in parallel to the SM rise. Obviously, further work is needed to understand the SM rise in algae.

5. THE M → T DECLINE

The M-to-T decline in *Chlorella* is strongly affected by the presence of the uncoupler FCCP (Papageorgiou and Govindjee, 1968a; Mohanty and Govindjee, 1974) and by the pH of the external medium, being slower at alkaline pH (Papageorgiou and Govindjee, 1971). Thus, it appears that the MT phase may be related to the synthesis and utilization of ATP in *Chlorella*. Of course, the effects of fluorescence yield are indirect (again, via structural changes of the membrane in which the pigment–protein complexes are embedded).

6. PHYSIOLOGICAL CHANGES

Chl *a* fluorescence transients are being extensively used as monitors of the physiological status of samples (also see Briantais *et al.*, Chapter 18, and Renger and Schreiber, Chapter 19, this volume). Because of the complexities of the various phases of the fluorescence transient, it is difficult to make meaningful interpretations, but in spite of this fluorescence can serve as a quick and sensitive indicator of changes in physiological parameters, which can then be investigated later by parallel measurements on partial reactions. We refer here only to two examples on green algae: the first deals with the effect of salts on *Chlorella* (Mohanty *et al.*, 1974) and *Chlamydomonas* (Wollman and Diner, 1980) and the second with the effect of the CO_2 concentration during growth of *Chlamydomonas* (Spalding *et al.*, 1984).

D. Flash-Induced Changes

Flash-induced Chl *a* fluorescence yield changes have provided important information on the primary photochemical and the associated reactions of PSII (see, e.g., Amesz and Duysens, 1977; Lavorel and Etienne, 1977; van Gorkom, 1985; and Chapter 10, this volume). For completeness, we shall mention a few key experiments on this topic on the green alga *Chlorella*.

The decay of Chl *a* fluorescence yield, after a 30-ms exciting flash, was monitored by Lavorel (1965), who showed two components of $t_{1/2}$ of ~10 ms and 0.1–1 s. The latter slow component was explained as due to a back reaction of Q_A^- with an oxidized component on the electron donor

side of PSII, an idea revived by van Best and Duysens (1975). The use of a shorter flash (microsecond range) allowed Zankel (1973) to resolve a fast ($t_{1/2}$ ~ 200 μs) component. This is now recognized as the $t_{1/2}$ of electron flow from Q_A^- to the second quinone acceptor, Q_B (for *Chlorella*, see, e.g., van Best and Duysens, 1975). The current electron transfer scheme on the acceptor side is

$$Q_AQ_B \xrightarrow{h\nu} Q_A^-Q_B \rightarrow Q_AQ_B^- \xrightarrow{h\nu} Q_A^-Q_B^- \longrightarrow Q_AQ_BH_2 \rightrightarrows Q_AQ_B$$
$$\uparrow H^+ \quad \uparrow H^+ \qquad\qquad PQ \quad PQH_2$$

Mauzerall (1972) measured the rise and the decay of the yield of Chl *a* fluorescence in *Chlorella* after 2-ns flashes. He observed that after the first flash, the fluorescence yield rise was in the 30-ns range, but after the second flash the rise was slower. Butler (1972) suggested that this rise was a measure of electron flow from the electron donor Z to $P680^+$ (the latter was proposed to be a quencher of Chl *a* fluorescence). (For an elegant and quantitative study, see Sonneveld *et al.*, 1979, also for *Chlorella*.)

The above discussion shows the versatility of Chl *a* fluorescence in monitoring electron flow from Z to $P680^+$ and from Q_A^- to Q_B in algal cells. In view of the known effects of various herbicides on the Q_A-to-Q_B reaction, the application of Chl *a* fluorescence to herbicide research by using algal cells as model systems has promising future possibilities.

E. Polarization

If polarized light is used to excite Chl *a* molecules, those with absorption dipoles parallel to the polarization of the incident light are preferentially excited. As energy is transferred among the Chl *a* molecules, the "memory" of the initial polarization (p) is lost if the Chl *a* molecules are arranged in a random fashion within the thylakoid membrane. (Note that the $p = (F_\parallel - F_\perp)/(F_\parallel + F_\perp)$, where $F_\parallel$ and $F_\perp$ are intensities of fluorescence parallel and perpendicular to the polarization of the exciting light, the direction of the measured fluorescence being perpendicular to the direction of the exciting beam.) In the discussion of fluorescence polarization, we should make a clear distinction between oriented and unoriented samples (see Amesz and Vasmel, Chapter 15, this volume). In the first case, with polarized excitation, one obtains information on the angles between exciting and emitting dipoles, but mixed with depolarization by energy transfer, as noted above. In the second case, with nonpolarized excitation, one learns about the orientation of emission dipoles with respect to the membrane; however, with polarized

excitation, the excitation spectrum gives information on the orientation of absorption dipoles. Mixed cases are difficult to analyze.

The earlier studies on unoriented samples showed low degrees (0.01 to 0.06)* of polarization of fluorescence of Chl *a in vivo* (Arnold and Meek, 1956; Goedheer, 1957, 1966; Weber, 1958; Teale, 1960; Govindjee, 1966; Cederstrand and Govindjee, 1966) in contrast to that *in vitro* ($p = 0.40$) (Goedheer, 1957; Teale, 1960). These low values were taken as evidence of energy transfer. The value of p is even lower for the variable ($F_p - F_0$) fluorescence than for the F_0 level (Lavorel, 1964) in *Chlorella*. Mar and Govindjee (1972) showed that addition of DCMU, which inhibits the electron flow, also reduces p; this was taken, in a first approximation, as evidence for further excitation migration in *Chlorella* (for a detailed and elegant study, see Whitmarsh and Levine, 1974).

In chloroplasts from higher plants, support for cation-induced changes in excitation energy distribution and redistribution between the two photosystems has come from observations of the cation-induced changes in the degree of polarization of Chl *a* fluorescence in unoriented samples (Wong *et al.*, 1979; Wong and Govindjee, 1981). Similar experiments on polarization of fluorescence are needed to study state changes in algae (see Section IV).

Light-harvesting Chl *a*/Chl *b* proteins from higher plants have been examined by Van Metter (1977a,b) for polarization of fluorescence. The small value (+0.02) observed for p on excitation at 650 nm (in Chl *b*) is indicative of a nearly spherical symmetry for the exciton states of the three Chl *b* molecules present there. A model was proposed (Knox and Van Metter, 1979), based on data on p as well as circular dichroism and absorption spectra, for the arrangement of the three Chl *a* and three Chl *b* molecules in LHCP. This model has been refined by Shepanski and Knox (1981). The basic picture is that the three Chl *a* molecules are arranged at the periphery of a core of three Chl *b* molecules whose dipoles are not parallel to each other. We suspect that the LHCP of green algae (see Section II,C) will show similar results.

During the 1970s several papers appeared on polarized fluorescence of oriented photosynthetic systems (Geacintov *et al.*, 1971, 1972, 1974; Breton *et al.*, 1973; Breton, 1975; Becker *et al.*, 1976; Garab and Breton, 1976), but the conclusions regarding the orientation of the various pigments are quite complex (see Breton and Vermeglio, 1982, for a summary, and consult the original papers for details). Using *Chlorella* cells oriented in a magnetic field (Geacintov *et al.*, 1971), Geacintov *et al.* (1974) were able to show that the ratio (FP) of fluorescence polarized parallel to that polarized perpendicular to the thylakoid membrane in *Chlorella* ranges from 1.2 to 1.9, especially at wavelengths >690 nm; this ratio is, however, closer to 1.0 at wavelengths <690 nm. The picture has emerged that the Q_y absorbance bands of the long-wavelength forms of Chl *a* (e.g., Chl *a* 695–712) are oriented approximately parallel to the plane of the membrane, that of Chl *a* 680 is also oriented somewhat (<30°C) parallel, whereas that of Chl *a* 670 is little oriented. Furthermore, the pigment responsible for F695 (at 77°K) is oriented more or less perpendicular to the membrane plane. (For a polarization study of magnetically oriented *Chlorella vulgaris* cells at 77°K, see Vasin and Verkhoturov, 1979.)

Gulyayev *et al.* (1982) found that the maximum polarization of fluorescence at room temperature was for F685, followed by F705 and then F680. However, also at room temperature, these authors observed that the polarization of fluorescence was low for F_0 and high for variable fluorescence. In unoriented samples, the reverse is true (Lavorel, 1964; Mar and Govindjee, 1972). Further research is needed to understand these different results. If it is proved that Chl *a* is significantly oriented at room temperature *in vivo*, then energy transfer will not cause complete depolarization of fluorescence, as was inferred in earlier studies (*vide supra*).

An application of measurements of polarization of fluorescence to the physiology of algal cells (i.e., effects of growth cycle) may be found in a paper by Chemeris and Venediktov (1980).

IV. State I–State II Changes

Bonaventura and Myers (1969), working on the green alga *Chlorella*, and Murata (1969), working on the red alga *Porphyridium*, reported light-driven changes in the distribution of excitation energy between PSI and PSII. Bonaventura and Myers (1969) coined the term (light) state I as the state created by exposure to light I (i.e., light preferentially absorbed by PSI) and (light) state II as the state created by exposure to light II. State I is recognized by a higher Chl *a* fluorescence yield at room temperature, a higher ratio of F685 and F695 to F720 at 77°K, and a higher quantum yield of PSII reactions at low light intensities. Conversely, state II is recognized by a lower Chl *a* fluorescence yield at room temperature, a lower ratio of F685 and F695 to F720 at 77°K, a lower

* J. Amesz (personal communication) suggests that these low values are due partly to overlap of absorption and emission bands at room temperature, and that low-temperature data, where the overlap is minimal, should give better results (cf. Kramer and Amesz, 1982, for studies on oriented spinach chloroplasts).

quantum yield of PSII, and a higher quantum yield of PSI reactions. For studies on state I–state II transitions in green algae, see Salamon (1980), Williams *et al.* (1980), Saito *et al.* (1983), Sane *et al.* (1982), and Hodges and Barber (1983). In the case of chloroplasts from higher plants, these state changes are linked to the phosphorylation of LHC II (see Briantais *et al.*, Chapter 18, this volume); for a discussion of state changes in phycobilin-containing algae, see Fork and Mohanty (Chapter 16, this volume).

Light I and light II induced changes in Chl *a* fluorescence properties, which were unrelated to the Q_A hypothesis of Duysens and Sweers (1963), were recognized earlier in algae by Papageorgiou and Govindjee (1967, 1968a,b). They were interpreted in terms of conformational changes in the membrane leading to changes in the orientation/distance between different Chl *a* molecules; the increase in the intensity of the S level by PSII preillumination (Munday and Govindjee, 1969a,b) was a manifestation of a similar phenomenon.

A redistribution of energy between PSII and PSI could cause the system to become more or less balanced and thus change the interaction of PSI light with Chl *a* fluorescence from PSII, explaining the different quenching efficiencies at different times of the transient (Mohanty *et al.*, 1970). Canaani *et al.* (1984) have shown clearly that in leaves of higher plants both the quenching of Chl *a* fluorescence by PSI light and the Emerson enhancement effect in O_2 evolution are high only in state I; in state II, the system is well balanced. [These results could explain the "old" controversies between investigators who could observe or not observe Emerson enhancement in the same experimental system; see discussions in Govindjee and Govindjee (1975) and Govindjee and Whitmarsh (1982).]

The state I → state II transition has been described (see, e.g., Allen *et al.*, 1981; see Fig. 7, Briantais *et al.*, Chapter 18, this volume) in terms of the following sequence: PSII light accumulates PQH_2; a kinase is activated; phosphorylation of LHC II occurs; phosphorylated LHC moves from a stacked to an unstacked region; and a balanced absorption cross section of PSI and PSII is achieved. Conversely, the state II → state I transition is described as follows: PSI light oxidizes the PQ pool; a phosphatase is activated; dephosphorylation of LHC II occurs; LHC II moves back to the appressed regions; and an unbalanced absorption cross section of PSII and PSI is attained. In addition to the above phenomenon, green algae show changes in excitation energy distribution or redistribution on dark adaptation (see, e.g., Catt *et al.*, 1984). Both the dark state and state II are poised in favor of PSI, whereas state I is poised in favor of PSII (see, e.g., Williams *et al.*, 1980; Satoh and Fork, 1983; Catt *et al.*, 1984). However, it was suggested that the dark state is different from state II. Catt *et al.* showed that low intensities of light II have a similar effect to light I (also see earlier observations of Munday and Govindjee, 1969b); i.e., the dark state is driven to an intermediate state between the dark state and state I. This low-intensity wavelength-independent change in green algae has been attributed to changes in the local ionic environment, while the high-intensity induced state I–state II changes are related to phosphorylation of LHC II (Catt *et al.*, 1984).

The state I → state II transitions and their relationship to phosphorylation of LHC II has been shown by Wollmann and Delepelaire (1984) in *Chlamydomonas* thylakoids and suggested by Saito *et al.* (1983) in *Chlorella* thylakoids. Further work is needed to prove that state transitions are related to LHC II phosphorylation in intact algal cells, although the current data favor this mechanism. The only experiment to date relating state changes to phosphorylation of LHC II *in vivo* is that of Canaani *et al.* (1984) on leaves, but even this conclusion is dependent on acceptance of the idea that sodium fluoride treatment of leaves specifically affected the LHC II phosphorylation in their system.

For a complete review on state changes, see Fork and Satoh (1986).

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Note Added in Proof

Evidence against the idea that variable fluorescence is delayed fluorescence is mounting: (1) Using an instrument with a 60-ps response time, Moya *et al.* (1987) were unable to confirm the results of Mauzerall (1985) in *Chlamydomonas;* in addition, these authors observed the presence of the slow component in a *Chlamydomonas* mutant lacking the reaction center II. (2) On the basis of an analysis of a model correlating the exciton decay kinetics in picosecond fluorescence studies with the primary processes of charge separation in the reaction center of PSII, Schatz and Holzwarth (1987) have also concluded that variable fluorescence is prompt, not delayed, fluorescence.

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