Chlorophyll

The generic name for the green pigments which are the photoreceptors of light energy in photosynthesis. These pigments belong to the tetrapyrrole family of organic compounds, which includes the open-chain bile pigments and the large ring compounds. The large-ring compounds are composed of porphyrins, dihydro- and tetrahydroporphyrins, as well as their derivatives chelated with metals such as iron (Fe) or magnesium (Mg).

Occurrence. Five closely related chlorophylls, designated *a* through *e*, occur in higher plants and algae. Vascular green plants contain chlorophylls *a* and *b* in the ratio of about 3 to 1. Other chlorophylls when present are in small or trace amounts. Trace amounts of biochemical precursors of chlorophyll, for example protochlorophyll and Mg protoporphyrin, are found under certain conditions. In certain algae, open-chain metal-free tetrapyrrole pigments called phycobilins are found attached to proteins. Photosynthetic bacteria form bacteriochlorophyll; the *Chlorobium* bacteria form chlorobium chlorophyll. *See* ALGAE; BACTERIA; BACTERIAL PHOTOSYNTHESIS; BACTERIAL PIGMENTATION; PHYCOBILIN.

In higher plants the chlorophylls and the above-mentioned pigments are contained in lipoprotein bodies, the plastids. At the highest magnification of the light microscope one may just see tiny grana in the plastids of higher plants. A granum is made up of 10–100 disks and resembles a stack of pennies. The disk, or thylakoid, is the basic photosynthetic apparatus and may be thought of as a flattened balloon; its continuous membrane is 50–80 A thick and it encloses a space about 80 A thick. The outer portion of the membrane differs in structure and function from the inner portion, but the exact molecular organization is not known. Within the membrane are the chlorophylls, which constitute 10% of the dry weight of the membrane, and two kinds of photosynthetic units, photosystem I and II. A photosynthetic unit is made up of a packet of enzymes together with several hundred chlorophyll molecules and carotenoid molecules. *See* CAROTENOID; CELL PLASTIDS.

Photosystem II absorbs shorter wavelengths of light than I and is thought to contain a high ratio of chlorophyll *b* to *a*. Photosystem I absorbs longer wavelengths and contains a high ratio of *a* to *b*.

Functions. Chlorophyll molecules have three functions: They serve as antennae to absorb light quanta; they transmit this energy from one chlorophyll to another over distances usually of 15–20 A by a process of "resonance transfer," so that the energy finally comes to reside in a chlorophyll molecule, P 695, in the receptor site of photosystem I or II; and finally, this chlorophyll molecule, in close association with enzymes, undergoes a chemical oxidation; that is, an electron of high potential is ejected from the molecule; this electron can then be made to do chemical work. In this way the energy of light quanta is converted into chemical energy. *See* PHOTOSYNTHESIS.

Chemistry. The structure of chlorophyll *a* is shown in Fig. 1. R. B. Woodward and coworkers

succeeded in synthesizing this molecule in 1960. The characteristic features of chlorophyll *a* are that it is a magnesium chelate of a dihydroporphyrin with a cyclopentanone ring (E) and is esterified with phytol. Chlorophyll *a* consists of four 5-membered rings (A-D) which form part of the large macro ring. Rings A-C are pyrrolic nuclei, whereas ring D is a dihydropyrrolic nucleus, that is, containing two extra hydrogen atoms. In the 6 position is an oxidized propionic acid group esterified with methanol which forms the cyclopentanone ring E. This ring contains the carbonyl oxygen at position 9 and the enolizable hydrogen atom at 10. The small rings are linked together through methine bridges $\overset{\diagup}{\underset{\diagdown}{\gg}}C\text{—}H$, α through δ, to form the large inner 16-membered ring of carbon and nitrogen atoms attached to each other through alternating single and double bonds.

The parent compound, porphyrin or porphin, consists of four pyrrole nuclei and is red, as in protoporphyrin. When a dihydropyrrole replaces one pyrrolic nucleus, the resulting compound is a dihydroporphyrin or chlorin and is green, as in chlorophyll. When two dihydropyrroles replace two pyrrolic nuclei, as in bacteriochlorophyll, the main absorption is in the far-red.

To get the structure of the following compounds, substitute in the chlorophyll *a* the atoms or groups in the position noted.

Chlorophyll *b*: in position 3, substitute —CHO for $—CH_3$.

Chlorophyll *c*: in position 7, substitute acrylic acid $—CH{=}CH—C(=O)—O$.

Chlorophyll *d*: in position 2, substitute —CHO.

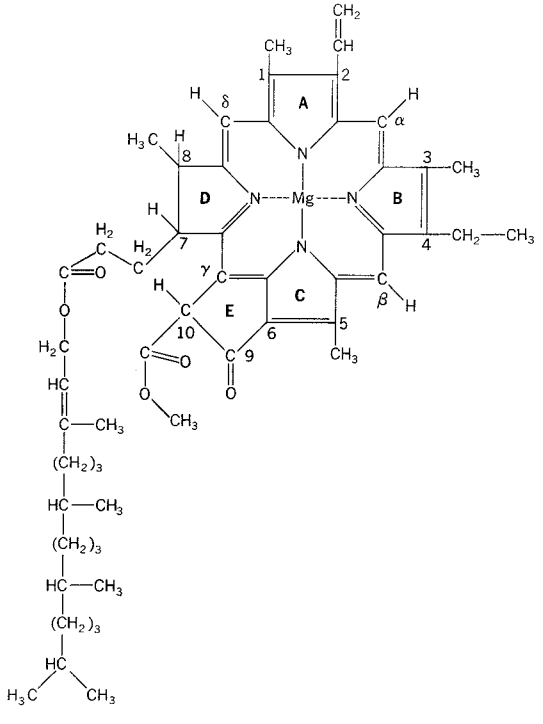

Fig. 1. Structure of chlorophyll *a* ($C_{55}H_{72}O_5N_4Mg$).

Bacteriochlorophyll: remove double bond between 3 and 4 and add an H atom at 3 and 4; at 2, substitute $—CO{\cdot}CH_3$.

Chlorobium chlorophyll 660: 2 is $—CHOHCH_3$; at 10, remove $COOCH_3$ and replace by H; at 7, farnesol (C_{15}) replaces phytol (C_{20}); at β or γ, a CH_3 or C_2H_5 replaces H.

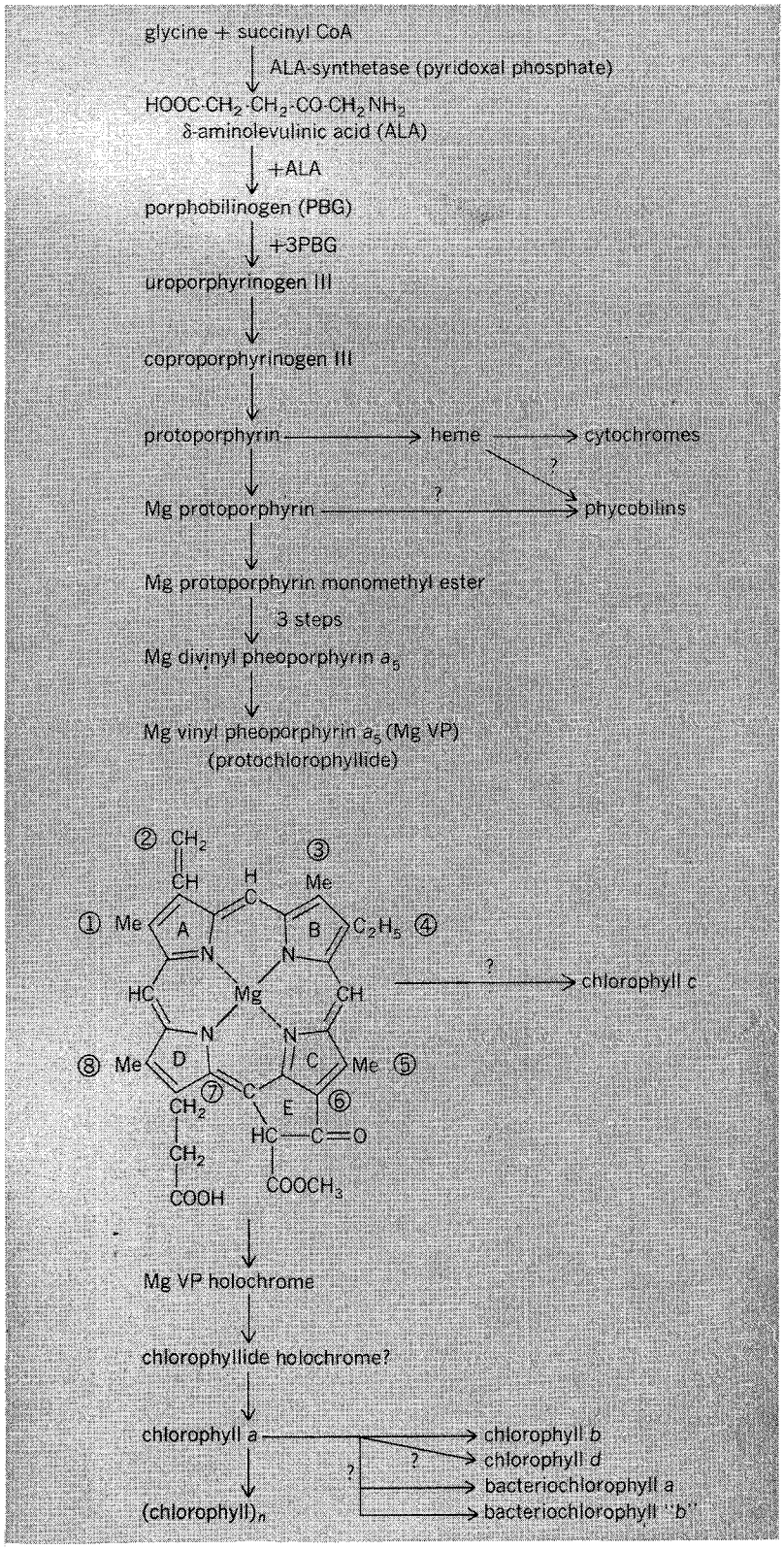

Fig. 2. Biosynthetic pathway for chlorophylls and some related compounds.

Pheophytin: remove Mg and add two H atoms on two of the N atoms.

Pheophorbid: remove Mg and phytol.

Protochlorophyll: in positions 7 and 8, remove H atoms and add a double bond.

Vinyl pheoporphyrin a_5: in positions 7 and 8, remove H atoms and add a double bond, also remove Mg and phytol.

Biosynthesis. The two major pigments of protoplasm, green chlorophyll and red heme, are synthesized along the same biosynthetic pathway to protoporphyrin, as shown by tracer and enzyme studies. Starting from the small building blocks, glycine and succinic acid, they are converted in a series of enzymic steps, identical in plants and animals, to protoporphyrin. Here the pathway branches to form (1) a series of porphyrins chelated with iron, as heme and related cytochrome pigments; and (2) a series of porphyrins chelated with magnesium which are precursors of chlorophyll (Fig. 2). *See* HEMOGLOBIN.

In plants it has not yet been demonstrated that δ-aminoleuvlinic acid (ALA) is formed from succinyl CoA and glycine by plastids. However, ALA has been shown to be converted by isolated *Euglena* plastids to protoporphyrin. ALA when added to etiolated leaves is converted to protochlorophyllide. Presumably all of the enzymes from ALA to chlorophyll are contained in the chloroplasts. In higher plants light and a special protein (holochrome) are required to convert protochlorophyllide to chlorophyllide; in this photoreaction two H atoms are added to the 7 and 8 positions of ring D. In a few plants, such as *Chlorella*, this reduction can occur enzymically and chloroplasts can be formed in the dark if glucose is supplied as a source of energy. Among additional factors required for chlorophyll formation are Fe, Mg, and O_2. Concomitant with chlorophyll synthesis, the disk, or thylakoid, membranes are also synthesized.

The possible origins of the phycobilin pigments, chlorophylls *b*, *c*, and *d*, and the bacteriochlorophylls are shown in Fig. 2.

Isolation and separation. Isolation of chlorophylls *a* and *b* consists in extracting leaves, such as nettles or spinach, which have little chlorophyllase, with 80% acetone containing some Na_2CO_3 or dilute NH_3 (to neutralize plant acids). Petroleum ether is added and the acetone is washed out with water, then with methanol to remove carotenols. Finally, the petroleum ether is washed free of acetone and methanol with water, which causes the chlorophyll to precipitate. It is filtered into a layer of talc, and the talc is washed with petroleum either to remove carotenes. The chlorophyll is then extracted with ether. The ether is dried with anhydrous Na_2SO_4, and chlorophyll is precipitated with petroleum ether. Separation of the chlorophylls, dissolved in a small volume of pyridine and diluted with petroleum ether, is achieved by chromatography on powdered sucrose or polyethylene columns. The column is developed with 0.5% isopropanol in pentane. Crystalline chlorophyll *a* is obtained by addition of water to an ether solution of chlorophyll which is slowly evaporated in vacuum. *See* CHROMATOGRAPHY.

Methods of column chromatography and paper chromatography have been described by L. P. Vernon and G. R. Sealy. The order of movement of the pigments is usually carotenes > pheophytin *a* > pheophytin *b* > chlorophyll *a* > lutein and zeoxanthin > chlorophyll *b* > violaxanthin > neoxanthin > pheophorbid *a* > pheophorbid *b* > vinyl pheoporphyrin. On the assumption that no pheophytins are present, chlorophylls $a + b$ can be estimated by O. Warburg's method of extraction of leaves with methanol, and the determination of the optical density (D) in a cell of 1-cm light path at 578 nanometers (nm), as shown in Eq. (1). For an ether solution J. H. C. Smith and A. Benitez give Eq. (2).

$$\frac{D_{578\,\mathrm{nm}}}{7.8} = \text{mg chlorophylls } a+b/\text{ml} \qquad (1)$$

$$\frac{D_{600\,\mathrm{nm}}}{9.95} = \text{mg chlorophylls } a+b/\text{ml} \qquad (2)$$

The method of G. Mackinney gives the concentrations of both chlorophylls $a + b$ in 80% acetone by solving the simultaneous Eqs. (3*a*) and (3*b*),

$$D_{663\mathrm{nm}} = 82.04\,C_a + 9.27\,C_b \qquad (3a)$$
$$D_{645\mathrm{nm}} = 16.75\,C_a + 45.6\,C_b \qquad (3b)$$

where C_a and C_b are the concentrations in milligrams per milliliter of chlorophylls *a* and *b*, respectively. [S. GRANICK]

Fluorescence. Chlorophylls, the important protagonists of plant and bacterial photosynthesis, reemit a fraction of the light energy they absorb as fluorescence. Irrespective of the wavelength of the absorbed light, the emitted fluorescence is always on the long wave-length side of the lowest energy absorption band, in the red or infrared region of the spectrum.

The fluorescent properties of a particular chlorophyll are functions of the structure of the molecule and its immediate environment. Thus, the fluorescence spectrum of a chlorophyll in the living plant is always shifted to longer wavelengths (peak at 685 nm) relative to the spectrum of a solution of the same pigment (peak at ~660 nm). This red shift is characteristic of aggregated chlorophyll.

Even in dilute solutions the capacity of chlorophyll to fluoresce depends on the nature of the solvent. In solvents which can combine with the central Mg atom of chlorophyll by donating a pair of electrons to it, chlorophyll is fluorescent. In solvents which lack this property, chlorophyll is dimeric and nonfluorescent at room temperature. The dimers are formed by combining the carbonyl group of one molecule with the Mg atom of the other.

The most widespread chlorophylls in nature, chlorophylls (Chl) *a* and *b*, fluoresce with a quantum efficiency of 0.33 and 0.16, respectively, in dilute solution in ethyl ether. In the living cell the quantum efficiency drops to 0.03 for Chl *a* and to zero for Chl *b*. This is due to the property of Chl *b* which transfers all its excitation to Chl *a*, which in turn channels most of its excitation to photosynthesis, allowing only a small fraction to escape as fluorescence.

An excited Chl *a* molecule in ethyl ether has a mean lifetime of five-billionths of a second, while in the living plant this is reduced to one- to two-billionths of a second. Long-lived excited states of Chl *a* (several thousandths of a second) have been observed under special conditions, such as illumi-

nation of concentrated solutions in dry hydrocarbon solvents at low temperatures. Under these conditions Chl *a* emits phosphorescence at a spectral maximum of 750 nm. *See* FLUORESCENCE COMPOUNDS, PLANT.

[GOVINDJEE; GEORGE PAPAGEORGIOU]

Bibliography: E. Rabinowitch, *Photosynthesis and Related Processes*, 1956; E. Rabinowitch and Govindjee, *Photosynthesis*, 1969; J. B. Thomas, *Primary Photoprocesses in Biology*, 1965; L. P. Vernon and G. R. Sealy (eds.), *The Chlorophylls*, 1966.

Fluorescence compounds, plant

Compounds which exhibit fluorescence in the ultraviolet to the infrared regions of the electromagnetic spectrum. The word fluorescence is derived from Latin words meaning "to flow" and "to yield." "Fluor" has been used for substances that can easily melt, but the term fluorescence is restricted to the transformation of a quantum of absorbed electromagnetic radiation into a quantum of emitted electromagnetic radiation usually of a longer wavelength (λ). (Luminescence, a more graphic term meaning to yield light, is used generally for all emissions of light.) The displacement of fluorescence bands toward the longer waves compared to the absorption bands is known as Stoke's shift.

A single molecule takes up each quantum, and the whole energy of the quantum is communicated to it. The absorbing molecule is thus excited, that is, lifted from its ground or normal state (E_0) of lowest energy and highest stability to excited energy-rich states E_1 or E_2 (Fig. 1*a*); this transition is extremely fast ($\sim 10^{-15}$ sec). The excited molecule has a very short life. For example, the lifetime

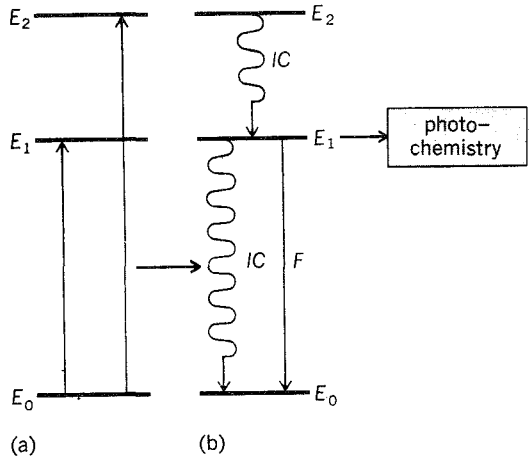

Fig. 1. Energy level diagram for a pigment molecule. (*a*) Excitation during absorption. (*b*) Return to ground state via fluorescence (*F*), internal conversion (*IC*), or photochemical reactions.

Absorption and fluorescence bands of photosynthetic pigments in the living cell*

Pigment	Absorption maxima, nm									Fluorescence maxima, nm
Chl *a*		436			676					685
Chl *b*		480			650					–
BChl *a*	376			590		800	850	890		900
BChl *b*		403		604				1017		1020-1050
CChl-650		446				730				771
CChl-660		457				750				770
C-Phycocyanin	380			620						650
R-Phycoerythrin		495	540	565						625
Allophycocyanin					654					665
C-Phycoerythrin			545	567						
B-Phycoerythrin			542	560						
	Ultraviolet	Blue	Green	Orange	Red	Near-infrared				Red to near-infrared

*The bands are indicated by the peak wavelengths in nanometers. Although the exact location of a maximum may vary somewhat from one photosynthetic organism to another, or sometimes a band may be missing entirely, the figures given are representative of the majority of the published data.

of the excited state of chlorophyll *a* in a living cell is about 1 nanosecond (10^{-9} sec).

The molecule in the excited state has several choices (Fig. 1*b*): (1) It may return to the ground state in one big jump, emitting a light quantum (fluorescence); (2) it may return to the ground state in various steps, releasing heat (internal conversion); or (3) the excess energy of the excited molecule may be utilized directly or indirectly for photochemical reactions such as photosynthesis. The following discussion concerns the fluorescence of pigments in plants and bacteria.

An enormous number of compounds capable of fluorescence occurs in the plant kingdom. Of these, the most studied compounds are those which play a role in photosynthesis and, particularly, the most conspicuously widespread organic compound on the surface of the Earth, the yellow-green pigment chlorophyll *a* (Chl *a*). *See* CHLOROPHYLL.

About 3% of light quanta absorbed by plants is reemitted as fluorescence within one-billionth of a second. A still smaller fraction of these absorbed quanta outlives this time limit by being stored temporarily, perhaps in the form of a metastable state of Chl *a*. Eventually, they are emitted as fluorescence, which is referred to as delayed fluorescence. This is distinguished from the ordinary prompt fluorescence, which claims the majority of the quanta fated to be emitted. The emission spectra of both the prompt and the delayed fluorescence are almost identical. Here only the prompt fluorescence is discussed.

Fluorescence is used extensively to unravel the mystery of the capture of light energy by the plant, and its subsequent storage in the form of energy-rich organic compounds. Fundamental problems, such as the immediate fate of the absorbed photon, the heterogeneity of chloroplast pigments, the association and the arrangement of the photosynthetic pigments in the living cell, the energy transfer from one pigment to the other in the living cell, and the kinetics of the reactions that occur immediately after the absorption of the photon, have been successfully dealt with by the application of fluorescence techniques. Such studies employ more than the fluorescence spectra (intensity of fluorescence as a function of wavelength) and the fluorescence excitation spectra (intensity of fluorescence produced by exciting with different wavelengths of light of equal incident quanta). They also include the time course of fluorescence (intensity of fluorescence as a function of time of illumination), the quantum yield (the number of emitted quanta per absorbed quanta), and the lifetime and the polarization of fluorescence. Since plant fluorescence refers primarily to the visible fluorescence of the photosynthetic pigments, this article will be confined to that class of plant compounds only. *See* BACTERIAL PHOTOSYNTHESIS; PHOTOSYNTHESIS.

The common pigment in all higher plants and photosynthetic algae is Chl *a*. In autotrophic bacteria, bacteriochlorophylls (BChl), compounds related to Chl *a*, play a similar role. In the plant cell, Chl *a* is accompanied by other colored molecules, the so-called accessory pigments. These include the green pigment chlorophyll *b* (Chl *b*) and minor chlorophylls, blue and red proteins, the phycobilins, and the yellow to orange carotenoids. These have absorption bands located between the two principal absorption bands of Chl *a*, in the blue and in the red region of the visible spectrum, thus enabling the plant to provide a wider "window" toward the Sun and to claim a greater share of the sunlight. Photons absorbed by the accessory pigments are transferred as electronic excitation energy to Chl *a*, where they are used in driving the primary reactions of photosynthesis. *See* CAROTENOID; PHYCOBILIN.

HIGHER PLANTS AND ALGAE

The absorption and fluorescence bands of the photosynthetic pigments in the living cell are shifted to longer wavelengths (red shift) in comparison to the spectral bands of the same pigments in solution. The principal absorption and fluorescence bands of the photosynthetic pigments in the liv-

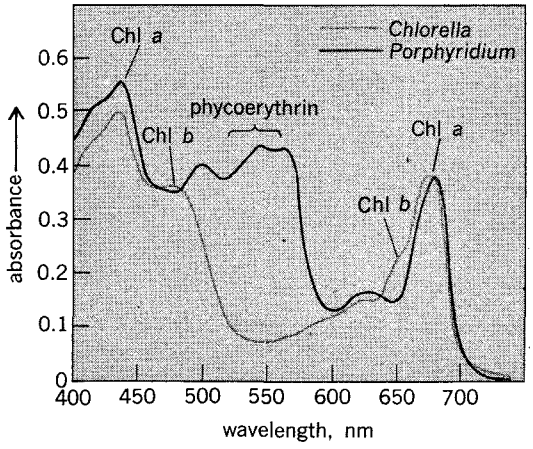

Fig. 2. Absorption spectra of a green alga (*Chlorella*) and of a red alga (*Porphyridium*), measured in an integrating sphere by a spectrophotometer. Absorption bands for chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and phycoerythrin are marked. (*From Govindjee, Transformation of light energy into chemical energy: Photochemical aspects of photosynthesis, Crop Sci., 7:551–560, 1967*)

ing cell are given in the table. The absorption bands of a diethyl ether solution of Chl *a* can be written as A_{410}, A_{430}, A_{578}, A_{615}, and A_{662} and the fluorescence band as F_{669}. In these notations, A and F refer to the absorption and fluorescence bands, respectively, with the subscript referring to the peak wavelength in millimicrons or nanometers (1 mμ or nm equals one-billionth of a meter). Of the absorption bands for the diethyl ether solution of Chl *a*, A_{430} and A_{662} are the most intense, so that the spectrum consists essentially of a blue (A_{430}) and a red (A_{662}) band. The principal absorption bands of Chl *a* in the living cell are A_{436} and A_{676}; the fluorescence band is F_{685} (Figs. 2 and 3). The fluorescence band is approximately a mirror image of the A_{676} absorption band.

Chlorophyll a. Although only one, unique Chl *a* molecule is extracted from the plant cell, there exists ample evidence that Chl *a* occurs in the living cell in the form of several spectroscopically and functionally distinguishable lipoprotein complexes or aggregates. Information for these Chl *a* forms was obtained from analysis of the red absorption and fluorescence bands mainly, since absorption by other plant pigments interferes with the Chl *a* absorption in the blue. At very low temperatures (−269 to −130°C), three bands of Chl *a* fluorescence are observed, with peaks at about 685, 696, and 720 nm (Fig. 4). These must originate from three distinct Chl *a* forms, since they can be selectively excited with light of certain wavelength and selectively destroyed by ultrasonic treatment of the plant cells. There are two pigment systems in plants, and it is known that a large part of the F_{720} fluorescence originates from a Chl *a* form belonging to system I of photosynthesis, while a large part of F_{685} and F_{696} bands are from system II Chl *a* forms. At ordinary temperatures the Chl *a* of system I is weakly fluorescent, with the bulk of fluorescence originating from the Chl *a* of system II.

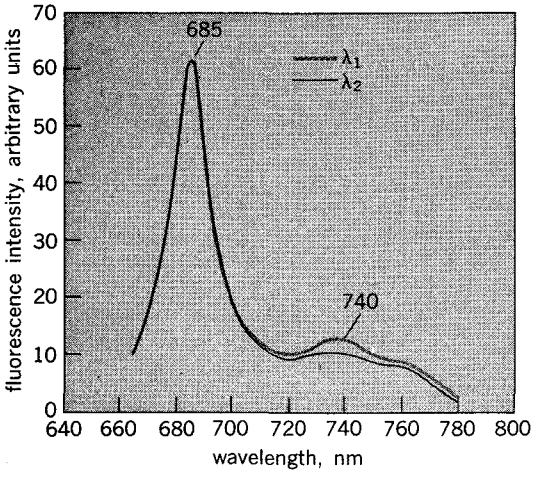

Fig. 3. Fluorescence spectra of chloroplast fragments from spinach upon excitation by two different wavelengths (λ_1, 635 nm; λ_2, 650 nm) of light. The measurements were made at 22°C. (*From Govindjee and L. Yang, Structure of the red fluorescence band in chloroplasts, J. Gen. Physiol., 49:763–780, 1966*)

Fluorescence polarization. Fluorescence excited by plane polarized light will be polarized if the molecules involved are uniformly oriented throughout the interval between absorption and emission. Weak fluorescence polarization indicates either motion of the excited molecules or exchange of the excitation energy among a system of randomly oriented molecules. Only weak fluorescence polarization has been detected in chloroplasts, and this is not influenced greatly by the temperature change. It appears, therefore, that efficient excitation energy migration from Chl *a* molecules to other Chl *a* molecules occurs in the living cell and is the cause of the weak polarization of fluorescence.

Fluorescence yield. Fluorescence and photosynthesis compete for the photons absorbed by Chl *a*, with photosynthesis taking by far the greater share. This competition makes possible the kinetic study of the fast photosynthetic reactions by following the time course of the fluorescence yield. The time courses of fluorescence and of the rate of oxygen evolution proceed first in a parallel and then in an antiparallel sense during the first few seconds of illumination. The parallel phase is interpreted to mean that an "activation" reaction exists, and the antiparallel part is there as required by the competitive relation between the two processes. On prolonged illumination, however, the fluorescence yield experiences a slow change, which is not reflected in a similar change in the rate of oxygen evolution. It is suggested by some investigators that this change originates from a light-induced slow alteration of the spatial arrangement of the Chl *a* molecules.

Accessory pigments. Light absorbed by the accessory pigments is transferred as electronic excitation to Chl *a*. As a result, the accessory pigments, with the exception of the phycobilins, do not fluoresce in the living cell. Furthermore, because of the excitation energy transfer, light absorbed by the plant results in Chl *a* fluorescence, irrespective of whether the absorption is carried out by Chl *a* itself or by the accessory pigments.

Accessory chlorophylls. Chlorophyll *b*, the main accessory pigment of higher plants and green algae, does not fluoresce in the living cell since it transfers all its electronic excitation to Chl *a*. The

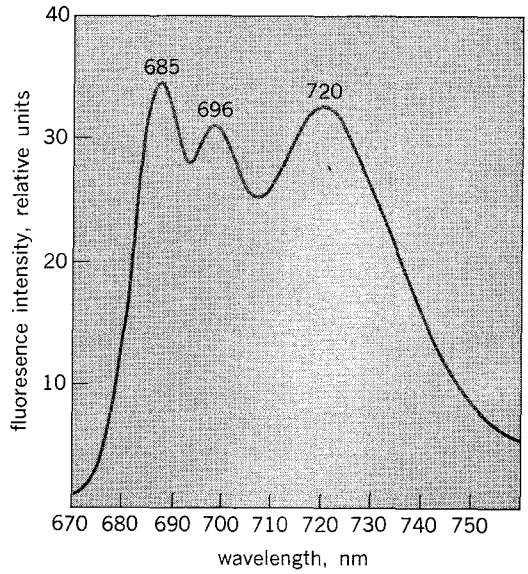

Fig. 4. Fluorescence spectra of the green alga *Chlorella pyrenoidosa* when cooled to −196°C. (*Courtesy of F. Cho and Govindjee*)

absorption bands of Chl *b* in a diethyl ether solution are A_{430}, A_{453}, A_{594}, and A_{642}, and the fluorescence band is F_{647}. Of these, A_{453} and A_{642} are the most intense. The fluorescence band is a mirror image of A_{642}. The principal absorption bands of Chl *b* in the living cell are A_{480} and A_{650} (Fig. 2).

Special chlorophylls occurring in minor quantities in some algae, but not in higher plants, are Chl *c*, Chl *d*, and Chl *e*. All are nonfluorescent in living cells.

Carotenoids. None of the various plant carotenoids is fluorescent. Nevertheless, a fraction of the light energy they absorb ends up, ultimately, in Chl *a*. The first experimental demonstration of excitation energy transfer between dissimilar molecules was carried out with brown algae (and diatoms), whose xanthophyll fucoxanthol was shown to transfer its excitation energy to Chl *a* and thus participate in photosynthesis.

Phycobilins. Phycobilins are colored proteins occurring in the red and blue-green algae and in marine microflagellates. Their molecule is made of a protein on which several tetrapyrrole chromophores (similar to those of the bile pigments) are linked covalently. The phycobilins are classified according to the color of their aqueous solutions as phycocyanins (blue) and phycoerythrins (red). In addition, prefixes C- and R- are employed to designate their origin from either the Cyanophyta (blue-green algae) or the Rhodophyta (red algae).

In contrast to other photosynthetic pigments, the phycobilins are water-soluble and fluorescent both in the test tube (Fig. 5) and in the living cell, the fluorescence yield being higher in the test tube.

In the plant cell, they must be located in close proximity to Chl *a* because they transfer their electronic excitation energy with high efficiency. The phycobilin content and the efficiency of the excitation energy transfer from phycobilins to Chl *a* depend on the intensity of the light used in culturing the algae.

The principal phycobilins are C-phycocyanin of the blue-green algae and R-phycoerythrin of the red algae. Other phycobilins are R-phycocyanin; C-phycoerythrin; allophycocyanin, which occurs in both red and blue-green algae; and B-phycocyanin, which is found only in *Smithora naiadum*. The absorption and fluorescence bands of the principal phycobilins are as follows: R-phycoerythrin, A_{495}, A_{540}, A_{565}, and F_{625}; C-phycocyanin, A_{380}, A_{620}, and F_{650}; and allophycocyanin, A_{654}, and F_{665}. A phycobilin transfers its electronic excitation energy either directly to Chl *a* or with the mediation of a second phycobilin, if the absorption band of the second phycobilin lies between the long wavelength absorption bands of the initial donor and the terminal acceptor Chl *a*. The weak polarization of the phycobilin fluorescence suggests that there is excitation energy migration among the tetrapyrrole chromophores of the same molecule.

PHOTOSYNTHETIC BACTERIA

A number of chlorophylls, structurally related to Chl *a* of higher plants, occur in the photosynthetic bacteria. By association with lipoproteins, each bacterial chlorophyll generates several, spectroscopically distinct forms in the living cell. These function both as primary and as accessory pigments in bacterial photosynthesis.

Bacteriochlorophyll a. This is the chlorophyll of purple bacteria, both Thiorhodaceae and Athiorhodaceae. Its principal spectral bands in ether solution are A_{358}, A_{392}, A_{577}, A_{773}, and F_{800}. In the living cell, association with lipoproteins (or aggregation) results in a red shift of the spectral bands and in a splitting of the A_{773} band into three components, located in the near-infrared. Thus, in most purple bacteria the spectral bands are A_{376}, A_{590}, A_{800}, A_{850}, A_{890}, and F_{906}. The relative height of the near infrared bands depends on the intensity of the light used for culturing; in some bacteria the A_{850} band is missing.

The fluorescence band corresponds to the longest wavelength absorption band. Light absorbed by BChl forms, whose bands are located at shorter wavelengths, is transferred as electronic excitation to the fluorescent form. Efficient transfer accounts for the inability to observe any fluorescence from

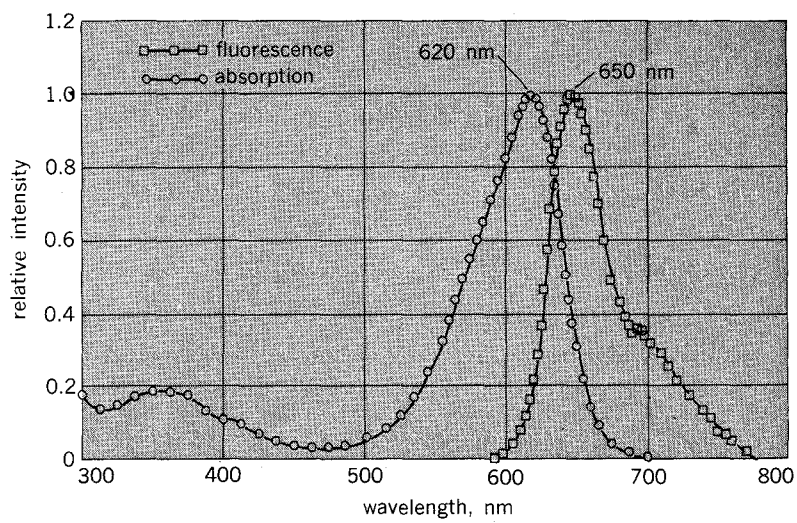

Fig. 5. Absorption and fluorescence spectra of a phycocyanin extracted from the blue-green alga *Anacystis nidulanis*. (*Courtesy of G. Papageorgiou*)

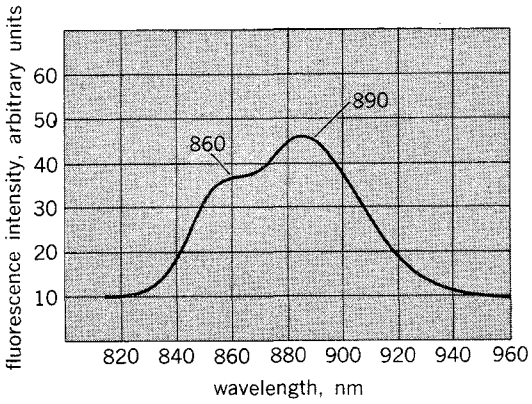

Fig. 6. Fluorescence spectrum of the *Rhodopseudomonas spheroides,* when excited at 520 nm. (*Courtesy of H. de Klerk and Govindjee*)

the short wavelength–absorbing forms of BChl *a*.

However, this transfer is not 100% in all cases, and fluorescence from the BChl form absorbing at 850 nm (A_{850}) has been observed (Fig. 6). Bacterial carotenoids also transfer part of their electronic excitation to the fluorescent form. *See* BACTERIAL PHOTOSYNTHESIS.

Bacteriochlorophyll b. Bacteriochlorophyll *b* has been identified in *Rhodopseudomonas viridis*. Its principal absorption bands in acetone solution are A_{368}, A_{582}, and A_{794}, with a minor band appearing at 675 nm. In the living cell, the spectral bands are A_{403}, A_{604}, A_{1017}, and $F_{1020-1050}$.

Chlorobium chlorophylls. These occur in the green photosynthetic bacteria, which contain in addition small quantities of BChl *a*. They have been classified according to the location of their longest wavelength absorption maximum of ether solutions as CChl-650 and CChl-660. These classes, however, do not correspond to unique molecular entities, each of them including several structurally distinct compounds. All are esters of farnesol instead of phytol, as is common in other chlorophylls. The members of the two classes differ in the substituents of the chlorin (7,8-dihydroporphin) ring.

Acetone solutions of the CChl-650 class have the following spectral bands: A_{406}, A_{425}, A_{557}, A_{605}, A_{651}, and F_{653}, of which the bands at 577 and 605 correspond to weak absorptions. The spectral bands of the CChl-660 class are, for acetone solutions, A_{413}, A_{432}, A_{627}, A_{662}, and F_{663}, with A_{627} weak. The principal absorption and fluorescence bands of the chlorobium chlorophylls in the living cell are, for CChl-650, A_{446}, A_{730}, and F_{771}; and, for CChl-660, A_{457}, A_{750}, and F_{770}. *See* FLUORESCENCE.

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