Chapter 9

The Photosynthetic Pigments

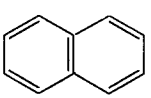
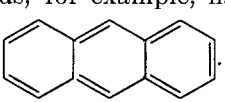
THE PIGMENT MOLECULES

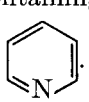
The most important components of the chloroplast lamellae are colored organic compounds—the photosynthetic pigments. Already Ingenhousz noted, in 1795, that colorless plant organs cannot "improve the air."

A rule, almost too obvious to deserve the title of the "first law of photochemistry" (which is sometimes bestowed on it), is that only light absorbed in a reacting system can have a chemical effect. However, when light absorption initiates a long chain of exergonic reactions, small, "catalytic" amounts of pigments may suffice. (An example is the photoinduction of flowering and other "photomorphological" effects, where the primary light-absorbing component proved very elusive, and was only recently identified as a colored protein, the "phytochrome.") Much larger amounts of pigments must be present to produce a sizable photochemical reaction when such chains cannot develop. A chain reaction is out of the question in photosynthesis because of the endergonic nature of the overall reaction, and yet photosynthesis must be a very fast process to provide all the organic material needed for the growth and life activity of the plant. This is why photosynthesizing cells have to contain very large amounts of pigments (up to 5% or more of total dry material).

Pigments are molecules strongly absorbing visible light. Such absorption is restricted to certain classes of compounds. In painting, we use

mostly the cheaper and more stable inorganic pigments (ochre, cobalt blue, cinnabar, chrome yellow, etc.); but good organic pigments have one or two orders of magnitude higher molecular absorption coefficients than the best inorganic ones.

The molecular structure of such organic pigments is characterized by long chains (or closed rings) of so-called "conjugated" double bonds. Carbon atoms, being quadrivalent, can be bound, in organic molecules, by single, double, or triple bonds, of which only single and double bonds are encountered in complex molecular structures. (The four bonds of a carbon atom are directed from the center to the four corners of a tetrahedron, and deflection from these directions needed to form a triple bond causes a stress.) Rings (or linear chains) consisting of regularly alternating ("conjugated") single and double bonds are particularly stable because they are reinforced by "resonance" (for example, that between the structures —C=C—C=C— and =C—C=C—C=)—interaction between several structures with the same arrangement of atoms, but different distribution of electrons. The most common of conjugated *rings* is that present in benzene, C_6H_6. It contains three single and three double bonds, and two main resonating structures are possible  and  (omitting the C and H symbols). More complicated rings of the same character are present in other "aromatic" compounds, for example, napthalene, $C_{10}H_8$,  and anthracene, $C_{14}H_{10}$,  . (Only one of the resonating structures is represented.)

The nitrogen atom, being trivalent, can form a single bond with one carbon atom, and a double bond with another; it can be, therefore, substituted for a carbon atom (or more precisely, for a CH-group) in conjugated systems. Rings containing atoms other than C are called *heterocyclic* (in contrast to the *homocyclic* rings, containing only carbon atoms). The simplest of them is pyridine, C_5H_5N, or  . One heterocyclic ring, common in plant pigments, is the five-membered pyrrol ring:  .

It is typical of molecules containing chains or rings of conjugated bonds to have strong absorption bands at relatively long waves, that

is, in the visible or in the near ultraviolet. (Organic molecules containing only single bonds, or isolated double bonds, usually absorb in the far ultraviolet.) As the number of conjugated double bonds increases, absorption shifts towards the longer waves, and becomes stronger. Thus, in benzene, the first absorption band lies below 250 nm, while in anthracene, it lies close to the visible, making this compound yellowish. In still more complex conjugated ring systems, such as those present in *indigo* or in *chlorophyll* (or in long linear conjugated chains, such as those found in *carotenoids*), the absorption bands shift into the visible, making these compounds pigments.

The strong light absorption by conjugated systems has to do with their resonance structures (of which the two above-mentioned energetically equivalent structures of benzene are the prototype). As shown in Fig. 9.1, the splitting of excited electronic energy levels into several

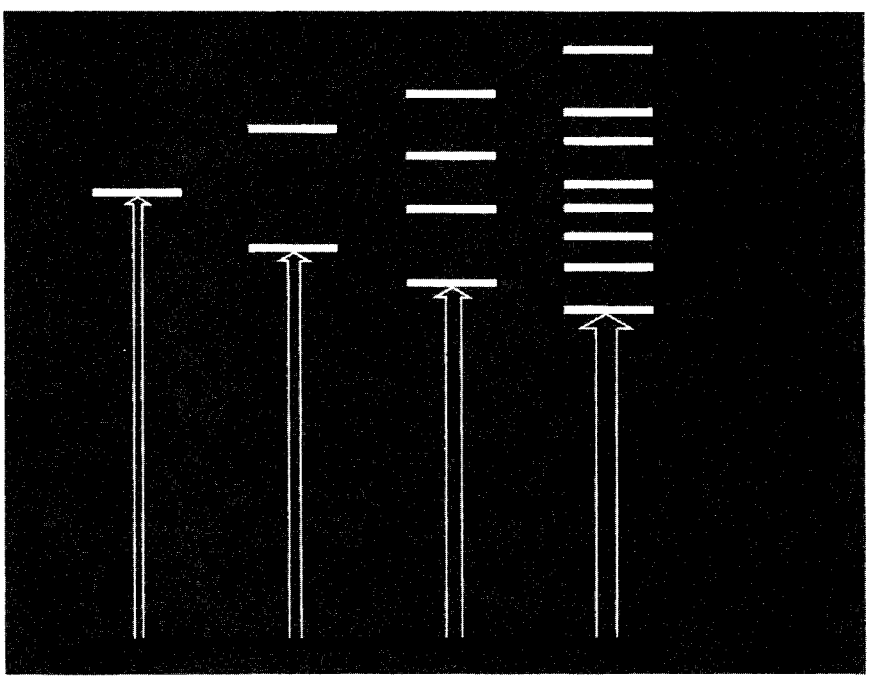


FIG. 9.1 Splitting of excited energy level by resonance in a chain of identical atoms (or molecules).

components, due to the existence of these resonating structures, leads to narrowing of the gap between the ground state and the first excited state; the first absorption band thus moves towards longer waves.

CHLOROPLAST PIGMENTS: GENERAL REMARKS

Photosynthetic organs of plants always contain an assortment of pigments. These can be divided into three major classes (see Tables 9.1–9.3): (1) chlorophylls and (2) carotenoids—both water insoluble, and (3) phycobilins—water-soluble (because of their attachment to water-soluble proteins).

The *first* general feature of the distribution of chloroplast pigments is the universal presence of *chlorophyll a*, found in *all* photosynthesizing cells (except photosynthetic bacteria, where a related pigment bacteriochlorophyll (BChl) is found instead). Chlorophyll *a* is present also in brown, red, and blue-green algae, where its green color is masked by other pigments. So far, no plant was found capable of true photosynthesis which did not contain chlorophyll *a* (Chl *a*). The widespread occurrence, as well as certain chemical properties of chlorophyll *a* in vivo (and of bacteriochlorophyll in bacteria), suggest that these pigments (or, at least some of their molecules) play an active role in photosynthesis, functioning as *photoenzymes*. All other pigments (and a large part of Chl *a* or BChl itself) seem to serve only as physical energy suppliers. Pigments other than Chl *a* are often called *accessory* pigments.

An enzyme is a protein catalyst that mediates a chemical reaction by active but reversible participation in it—as a middleman who buys a commodity from the seller to sell it to the buyer. The commodity, in the case of a redox reaction, is hydrogen atoms or electrons. A photoenzyme is a broker who can ply its trade only after having been activated by light absorption. Because of its role as a light-activated enzyme in photosynthesis, chlorophyll has been called "the most important organic compound on earth."

The *second* common, but not universal rule is the presence of a second kind of chlorophyll, in addition to Chl *a*. Higher plants and green algae contain, in addition to the yellow-green chlorophyll *a*, the blue-green

TABLE 9.1 The Chlorophylls

Type of chlorophyll	Characteristic absorption peaks		Occurrence
	In organic solvents, nm	In cells, nm	
Chl *a*	420, 660	435, 670–680 (several forms)	All photosynthesizing plants (except bacteria)
Chl *b*	453, 643	480, 650	Higher plants and green algae
Chl *c*	445, 625	Red band at 645	Diatoms and brown algae
Chl *d*	450, 690	Red band at 740	Reported in some red algae
Chlorobium chlorophyll (also called "bacterioviridin")	Two forms: (1) 425, 650 (2) 432, 660	Red band at 750 (or 760)	Green bacteria
Bacteriochlorophyll *a* (BChl*a*)	365, 605, 770	Red bands at 800, 850, and 890	Purple and green bacteria
Bacteriochlorophyll *b* (BChl*b*)	368, 582, 795	Red band at 1,017	Found in a strain of *Rhodopseudomonas*, (a purple bacterium)

chlorophyll b (Chl *b*)—a locally oxidized derivative of Chl *a*. Several other chlorophyll-type compounds have been described in the literature. *"Chlorophyll c"* is found in brown algae and diatoms. A "chlorophyll *d*," identified by H. Strain and others in red algae, has proved elusive in later experiments. A "chlorophyll *e*" has been reported in golden-yellow algae.

Green bacteria contain mostly so-called "Chlorobium chlorophyll," or *bacterioviridin*, in addition to traces of *bacteriochlorophyll*, which is the main pigment of purple bacteria.

TABLE 9.2 The Carotenoids

Types of carotenoids	Characteristic absorption peaks, nm[a]	Occurrence
	I. CAROTENES	
α-Carotene	In hexane, at 420, 440, 470	Many leaves, and certain algae. In red algae and a group of green algae called *Siphonales*, it is the major carotene
β-Carotene	In hexane, at 425, 450, 480	Main carotene of all other plants
γ-Carotene	In hexane, at 440, 460, 495	Major carotene of green sulfur bacteria; traces in some plants
	II. CAROTENOLS (also called "xanthophylls")	
Luteol	In ethanol, at 425, 445, 475	Major carotenol of green leaves, green algae and red algae
Violaxanthol	In ethanol, at 425, 450, 475	Second major carotenol of leaves
Fucoxanthol	In hexane, at 425, 450, 475	Major carotenol of diatoms and brown algae
Spirilloxanthol	In hexane, at 464, 490, 524	Common in purple bacteria

[a] It has been difficult to establish the exact location of carotenoid bands in vivo (except in the case of purple bacteria) because of their strong overlapping with the blue-violet bands of chlorophylls. The bands in vivo are estimated to be shifted by about 20 nm to the long-wave side from their position in solution. In the case of fucoxanthol, absorption extends in vivo to 550 nm.

TABLE 9.3 The Phycobilins

Types of phycobilins	Absorption peaks	Occurrence
Phycoerythrins	In water, and *in vivo:* 490, 546, and 576 nm	Main phycobilin in red algae, also found in some blue-green algae
Phycocyanins	At 618 nm, in water and *in vivo*	Main phycobilin of blue-green algae; also found in red algae
Allophycocyanin	At 654 nm in phosphate buffer at pH 6.5	Found in blue-green and red algae

An additional complication—to be discussed later in the chapter—arises from the apparent presence in living cells of several forms of one and the same pigment, which become identical upon extraction. This fact was long known in the case of bacteria, where three distinctly separated far-red absorption bands of bacteriochlorophyll are observed in vivo, at approximately 800, 850 and 890 nm, but give rise, upon extraction into an organic solvent, to a single absorption band at 770 nm. It has been surmised that bacteriochlorophyll is present in the living cell in the form of complexes with three different proteins, or in three different aggregation stages. A similar but less conspicuous polymorphism exists also in the case of chlorophyll *a* in green plants.

The *third* general rule, from which no exceptions are known, is the presence in all photosynthesizing cells of an assortment of carotenoids—relatives of the pigment that accounts for the orange color of carrot roots. Most carotenoids are yellow or orange. Their color is normally masked by chlorophyll, but in chlorophyll-deficient, so-called *aurea* varieties of plants, or in fall, when chlorophyll disintegrates, the yellow pigments become visible. Some of them become in autumn orange, or even red, through oxidation, thus contributing to the variety of foliage colors. (However, important contributions to the bright red or purple colors of some leaves in autumn are also made by pigments of another class, so-called anthocyanins.)

The specific assortment of carotenoids is different in plants of different classes. In general, there are two major groups: the *carotenes* (which are hydrocarbons) and the *carotenols*. The ending "ol" suggests that these compounds are mostly alcohols (although some of them are ketones). The plant carotenols are also designated as *xanthophylls* (from *xanthos* = orange-yellow).

Certain algae are brown; examples are large algae such as *Fucus*, familiar to all visitors on ocean beaches, and the free-swimming microscopic diatoms (*Bacillariophyceae*), having rigid silica skeletons. The diatoms are among the most successful plants on earth; they fill the upper layers of the oceans and may account for as much photosynthesis on earth as the green land plants. The brown color of all these organisms is due to a special carotenol, called *fucoxanthol*.

The *fourth* important fact is the presence, in the red marine algae (*Rhodophyceae*), and the primitive blue-green algae (*Cyanophyceae*, encountered on land as well as in shallow water), of pigments of a

still different class, called *phycobilins* (from *phycos* = alga, and *bilin* = bile pigment), because of their similarity to the pigments of the bile. These pigments may be present in amounts equal to, or greater than, those of chlorophyll *a*. Phycobilin is a generic name for pigments of two kinds: the red *phycoerythrins* (from *erythros* = brick red), and the blue phycocyanins (from *cyanos* = blue). The phycobilins are structurally related to chlorophylls (see below). The phycoerythrins appear to have the definite function of improving the light absorption in the middle of the visible spectrum; the same is probably true of the above-mentioned "brown" carotenoid, fucoxanthol.

Phycobilins can be extracted from the algae simply by placing the latter in distilled water, while chlorophyll extraction requires organic solvents. We have seen that chloroplasts contain hydrophilic proteins and hydrophobic lipoids; the latter, like grease spots, can be extracted only by organic solvents. As mentioned in Chapter 8, chlorophyll molecules have affinity to both polar and nonpolar molecules; this is why they can be most easily extracted by a mixture of organic solvents, such as acetone or alcohol, with water. The latter disintegrates the protein structure, while the former pulls out the lipoid molecules.

Phycobilins have no phytol chains and are attached to water-soluble proteins; they are thus easily extractable into pure water. Most carotenoids have no affinity for water and are soluble only in organic solvents.

CHLOROPHYLLS

Structure

The chlorophylls *a* and *b* have a common basic structure. Some familiarity with organic chemistry is needed to discuss it. It is a "porphyrin" structure, consisting of four pyrrol rings (Fig. 9.2), joined into a single master ring by CH "bridges." Figure 9.2 represents *porphin,* the mother substance of all porphyrins. Often a metal atom is found in the center of a porphyrin—iron in heme, magnesium in chlorophyll. In the latter, a long chain of carbon atoms ("phytol chain," from *phytos*=plant) is attached to the ring system. Other groups attached to the porphin skeleton will be discussed below. These side chains, and

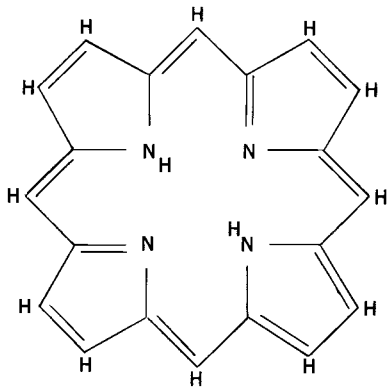


FIG. 9.2 Porphin structure consisting of four pyrrol rings (all corners except those marked N are occupied by carbon atoms).

the double bonds present in the overall structure, distinguish among themselves the numerous compounds of this class.

The porphin unit plays a very important role in nature. It forms the skeleton of both chlorophyll and heme, the red pigment of blood, and of other compounds of physiological significance (for example, the cytochromes).

The complete molecular structure of *chlorophyll a* is represented in Fig. 9.3. (Two Nobel prizes were awarded for the development of this structure, to the German chemists Richard Willstätter, and Hans Fisher, respectively; a third one was awarded to Robert B. Woodward of Harvard for total in vitro synthesis of chlorophyll.) Compared to the porphin structure of Fig. 9.2, that of chlorophyll *a* is characterized by a missing double bond in one of the pyrrol rings (ring IV). It is thus derived from a dihydroporphin (a porphin containing two additional hydrogen atoms).

Bacteriochlorophyll is derived from tetrahydroporphin, with *two* fewer double bonds and *four* more hydrogen atoms than porphin.

A peculiar and apparently important characteristic of chlorophyll (and of the bacteriolchlorophyll, too) is the presence of a fifth, "homocyclic," five-membered ring (ring V in Fig. 9.3) which carries a carbonyl group, C=O. This additional ring may be the "nerve center" of the chlorophyll

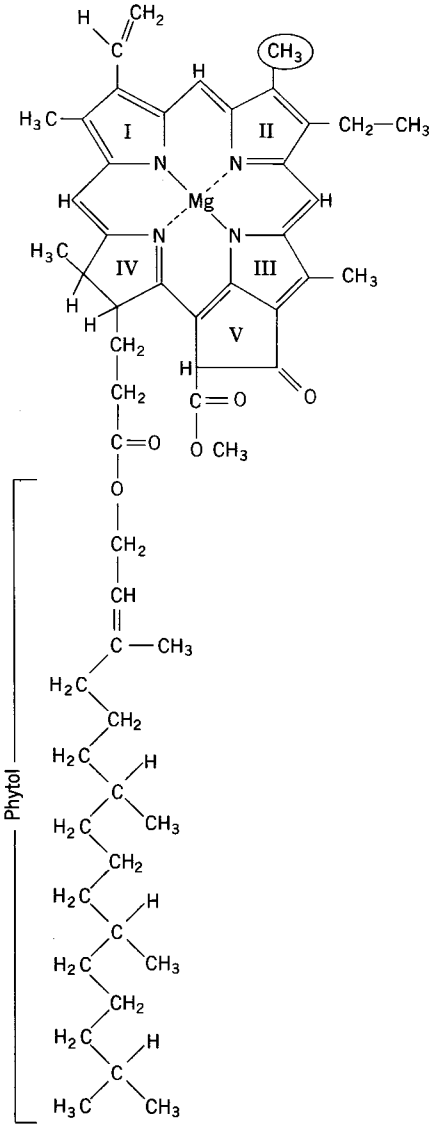


FIG. 9.3 Chlorophyll *a* (the circled CH_3 group is replaced by CHO group in chlorophyll *b*). All corners except those marked N are occupied by carbon atoms.

molecule, most closely associated with its photocatalytic action in photosynthesis. This conclusion is not based on direct evidence, but rather on general feeling arising from the totality of chemical and photochemical experience with chlorophyll and related compounds.

Another peculiarity of the chlorophyll molecule is the above-mentioned presence of a magnesium atom in the center of the molecule. We do not know its function. In hemin, the same position is occupied by an atom of iron, and the capacity of iron to exist in two oxidation states, as ferric ion, Fe^{+3} and ferrous ion, Fe^{+2}, gives a hint as to its possible catalytic function. Magnesium does not have a similar property, and its special appropriateness to serve as center of such a catalytically active molecule is not clear. (Of all commonly available metals, it may perhaps fit best into the available space!) The presence of a metal atom, with its tendency to assume a positive charge, pushes the electrons in the porphyrin ring towards the periphery of the molecule, and thus into ring V. This may be important for its catalytic properties. But, again, this is not a matter of clear-cut evidence, but of chemical "feeling."

Some short side chains in the chlorophyll molecule may be simply residues left over from its synthesis in the organism, which begins with short-chain molecules, such as those of acetic acid, CH_3COOH, and of glycine, NH_2CH_2COOH. When these are combined into rings, methyl groups, CH_3, are left hanging outside the ring. However, the presence in ring I of a nonsaturated (vinyl) side chain, $—CH{=}CH_2$, may be of some significance because its double bond is conjugated on one side with the ring system, and thus affects its overall properties. Also important is the presence, in ring IV, of the long and almost saturated phytol chain. (Phytol contains only one double bond, and fourteen single bonds.) This chain causes chlorophyll molecules to attach themselves to other saturated long-chain molecules in the chloroplasts, that is, it gives it *lipoid solubility* (lipoids being a generic name for "fatlike" compounds). The general rule in organic chemistry is that polar molecules associate with other polar molecules or groups (and are, therefore, attracted to water, which is the most polar of all simple solvents), while nonpolar chains associate selectively with other nonpolar chains. (In inorganic chemistry, on the other hand, the most important bonds involve *positive* ions associating themselves with *negative* ions, as in the formation of acids, alkalis, and salts.)

Chlorophyll *b*—the second variety of chlorophyll present in green plants and green algae—differs from chlorophyll *a* only by local oxidation of one side chain in ring II (marked by a ring in Fig. 9.3). This side chain is $-CH_3$ in Chl *a* and $-\overset{\overset{O}{\|}}{C}-H$ in Chl *b*.

Absorption Spectra

As a pigment, chlorophyll *a* is characterized by two strong absorption bands, located in the blue-violet and in the red region of the spectrum (Fig. 9.4*a*). The first of them, often called the *Soret band,* is common

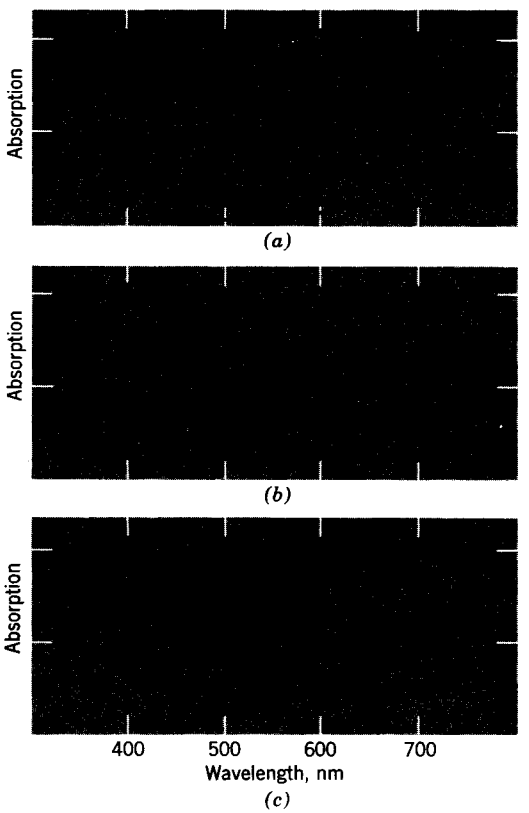


FIG. 9.4 Absorption spectra of three types of chloroplast pigments. (*a*) chlorophylls; (*b*) carotenoids; (*c*) phycoerythrins and phycocyanins.

to all porphin derivatives; but the second one is peculiar of chlorophyll and other compounds derived from dihydroporphine. In diethyl ether, the first has its maximum at 430 nm and the second at 660 nm. In the living cell, these bands lie at about 440 and 675 nm, respectively, and are complex (see last section of this chapter). The absorption is weak between the two bands, that is, in the green part of the spectrum, causing the green color of vegetation.

Chlorophyll *b* spectrum differs from that of Chl *a* by the two bands being closer together: the Soret band of Chl *b* is located (in ether) at 453 nm, and the red band at 643 nm. The ratio of intensities of the two bands is shifted in Chl *b* strongly in favor of the Soret band compared to chlorophyll *a*.

Bacteriochlorophyll *a* (BChl *a*) also has two main bands, but they lie farther apart; the Soret band in the near ultraviolet (at 365 nm) and the long-wave band, at the limit of the visible, at 770 nm (in methanol). In the living cell, the same bands lie at about 367 and 800–900 nm. The second one is usually split into three components, at about 800, 850, and 890 nm. A bacteriochlorophyll *b* has been recently discovered in some bacteria; it has an infrared absorption band at 1014 nm. Another chlorophyll derivative (mentioned before) is found in green bacteria; it is the "Chlorobium chlorophyll" or "bacterioviridin." In the living cell, the long-wave bands of this chlorophyll are found at 750 or 760 nm. Chlorobium chlorophyll-carrying green bacteria always contain also small quantities of bacteriochlorophyll *a*.

Chromatic Adaptation

The presence of a strong, long-wave absorption band, covering much of the red and orange region, in which sunlight is most abundant, may be one of the reasons why chlorophyll has been selected by nature as the main pigment in photosynthesis. Without this absorption band, the parts of the solar spectrum that contain the greatest amounts of useful energy would not be efficiently absorbed by cells. On the other hand, the specific form of the absorption spectrum of chlorophyll, with its absorption gap in the green region, which accounts for the pleasant green color of vegetation, implies a low capacity of these plants to utilize light in the middle of the visible spectrum. This deficiency is made up, at least to some extent, by the development of tiered vegetation: one tier of leaves lets enough light through for a second and a third

layer to survive. In deciduous forests, bushes and grass can live on the ground. (Coniferous forests, on the other hand, often have such dense crowns of needles that the shadow under them is too deep for any vegetation to develop on the ground.)

The position of the absorption bands in the red cannot be the only reason why chlorophyll *a* had been selected by evolution as *the* photocatalyst in photosynthesis. Other pigments exist that would provide a better coverage of the visible spectrum. Additional reasons for this selection must be sought in specific *physicochemical* properties that bind chlorophyll in appropriate locations in the cell; and even more, in specific *catalytic* properties, which permit it to serve as an effective photocatalyst in photosynthesis. The selection of chlorophyll may be the best solution nature found to satisfy two needs: (1) for effective absorption of visible light, and (2) for proper photocatalytic properties.

As mentioned above, green land plants and green algae contain chlorophyll *b* in addition to chlorophyll *a*. Because of the shift of its Soret band, chlorophyll *b* in solution is blue-green, while chlorophyll *a* is yellowish green. The presence of chlorophyll *b*, therefore, narrows somewhat the "green gap" in the absorption spectrum of leaves; this may be the rationale for its presence, in relatively larger quantities, in shade-adapted plants.

We have also encountered another method of plant adaptation to life under water. The brown algae, as well as the diatoms, narrow the green gap by the provision of two other pigments—chlorophyll *c*, (whose chemical nature is not yet well known; it may be a porphin rather than a chlorin derivative), and of a specific carotenoid pigment, fucoxanthol (to be discussed below). Even more effective in filling the "green gap" is phycoerythrin, the main pigment of red algae (cf. below).

PHYCOBILINS (PHYCOERYTHRINS AND PHYCOCYANINS)

Structure

The chemical structure of the phycobilins is related to that of chlorophylls. If one snips the ring system of chlorophyll and permits the four pyrrol rings to straighten out, the magnesium atom slips out and what remains is an open conjugated system of four pyrrol rings. This is the

fundamental structure of the *bilin* pigments, which can be considered as derived from a common parent, the hydrocarbon *bilan* (see Fig. 9.5*a* and *b*). The bilins are so called because they were first discovered in bile, to which they give its dark color. They may be, in fact, products of metabolic transformation of hemoglobin and chlorophyll, ingested with animal and plant food. The name phycobilin means algal bilin. Recently, it has been shown that the pigment phytochrome, responsible for the light control of seed germination and of flowering, is chemically related to the phycobilins.

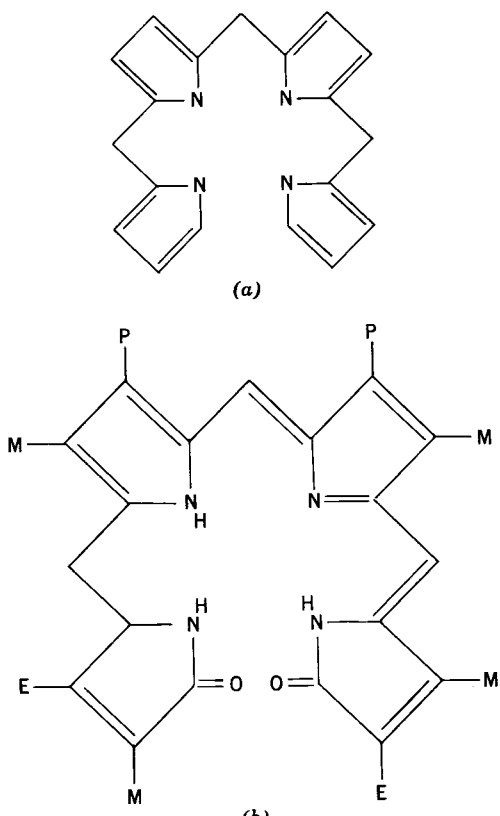


FIG. 9.5 Structure of bilin pigments. (*a*) bilan; (*b*) phycoerythrobilin. (All unmarked corners are occupied by carbon atoms.) (E, ethyl; M, methyl; P, propionyl groups.)

Absorption Spectra

In algae living deep under the sea, the deficiencies of chlorophyll as a light absorber become critical because light reaching these organisms is filtered through thick greenish-blue layers of water. (Dissolved salts, and organisms living in the surface layers, contribute to the absorption of red and blue-violet light.) Plants living under those conditions have evolved auxiliary pigments that absorb green light. Red algae, in particular, have solved the difficulty by means of a phycobilin called phycoerythrin, with absorption bands in the middle of the visible spectrum. For example, one type of phycoerythrin has bands at about 500, 545 and 570 nm (Fig. 9.4*c*). This permits these algae to perform photosynthesis (and thus to grow) in dim blue-green light prevailing in the depths of the ocean. Together, chlorophyll and phycoerythrin let through only a band in the far-red. This is why the algae which contain phycoerythrin appear red.

The deeper under the sea a red alga lives, the more phycoerythrin it contains in relation to chlorophyll.

However, complications make the overall picture more complex. For example, some algae encountered in great depth are green. They manage to survive by slowing down their life processes sufficiently to permit the small amount of light that their green pigment is able to catch to cover their needs.

Some surface, or even terrestrial, algae contain a pigment related to phycoerythrin, called *phycocyanin*. This is blue rather than red because its main absorption band is located at about 630 nm (Fig. 9.4*c*). It does not fill up the gap in the chlorophyll *a* spectrum, but it narrows it, similarly to Chl *b*. The algae that contain this pigment are bluish-green (*Cyanophyceae*). They are primitive organisms, intermediates between true plants and bacteria. Like bacteria, the blue-green algae do not have photosynthetic organelles bounded by a membrane (chloroplasts); like green bacteria, they contain a pigment that absorbs at 750 nm. It can be surmised that primitive plants of the type of blue-green algae have later developed in two directions—one in which the phycobilins have disappeared, and one in which one particular form of phycobilin, the phycoerythrin, has accumulated. Plants of the first type have taken over land and the surface layers of the ocean, while plants of the second kind have concentrated in the depths of the ocean. How-

ever, the division is not sharp, with some species of one kind invading the domain of the other.

CAROTENOIDS

Structure

The third type of pigments, present in all photosynthesizing cells, the carotenoids, contain an open-chain conjugated double bond system of so-called polyene type (Fig. 9.6), ending with so-called "ionone" rings. Carotenoids are either hydrocarbons, in which case they are called carotenes, or oxygen-containing compounds, in which case they are called carotenols (or xanthophylls). The three common carotenes are α, β, and γ carotene. They are "stereoisomers" having the same molecular formula $C_{40}H_{56}$ (Fig. 9.6), and distinguished only by the arrangement of their molecules in space. The carotenols have their oxygen in the form of hydroxyl, carbonyl, or carboxyl groups attached to the "ionone" rings.

Absorption Spectra

The carotenoids generally are *yellow* or orange; they have a group of two or three absorption bands located in the blue-violet part of the spectrum (Fig. 9.4*b*). For example, β-carotene, dissolved in hexane, has absorption bands at 430, 450 and at 480 nm. Certain algae—diatoms (*Bacillariophyceae*) and brown algae (*Phaeophyceae*, from *phaeos* = brown)—contain abundantly a special carotenoid, called *fucoxanthol*. In the form in which it is present in the cells, fucoxanthol has a much broader absorption band than other carotenoids; it absorbs not only in the blue but also in the green part of the spectrum. Instead of falling

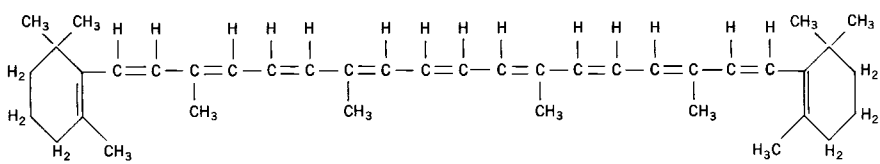


FIG. 9.6 Structure of β carotene (α and γ forms are stereoisomers). (All corners on the rings at the two ends are occupied by carbon atoms.)

off sharply to zero at about 500 nm, as in Fig. 9.4*b*, the blue absorption band of fucoxanthol declines slowly, covering much of the gap left by chlorophyll in the green, and destroying the pure green color of the plant.

To sum up, plants have developed, for the needs of photosynthesis, not a single pigment, but various pigment assortments. These change from one phylum of plants to another. The relative amounts of the several components change not only from phylum to phylum, or species to species, but also from specimen to specimen—from a shade-adapted to a sun-adapted leaf on the same tree, from a young to an old algal cell, from a specimen grown in green light to a specimen grown in red light, etc. The varying ratios of different pigments in the living cell reflect a dynamic balance of continuous synthesis and decomposition of the pigments.

It has been long a subject of guessing why chloroplast pigments occur in pairs, such as chlorophyll *a* and chlorophyll *b*, phycoerythrin and phycocyanin (a little phycocyanin is always found in red algae), and carotenes and carotenols. One member of the pair is slightly more oxidized than the other. So far, no explanation exists.

MULTIPLICITY OF CHLOROPHYLL *a* FORMS IN VIVO

Looking at the red absorption band of chlorophyll *a* in the living cell (Fig. 8.12*c*), one notes its greater width compared to the corresponding band in solution. (The "half-width" of the band—that is, its width at the level corresponding to half its maximum intensity—is 30 nm in vivo, as against about 18 nm in organic solvents.) Recently, careful observations suggested that the width of the red band in vivo is due to the presence of at least two and probably three (or even four) components.

The analysis of the absorption bands of chlorophyll *a* in suspensions of chloroplasts, or in whole algal cells, encounters several difficulties.

1. Other pigments besides chlorophyll *a* are present; this makes, in particular, the analysis of the Soret band almost impossible.
2. Light is strongly scattered by the suspension. There is general

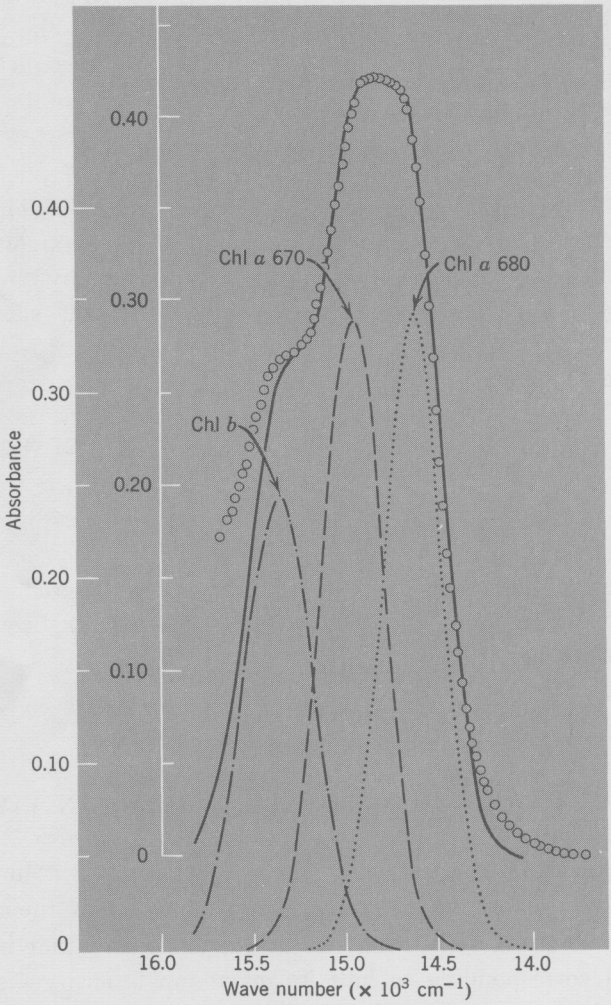


FIG. 9.7 Analysis of red chlorophyll absorption band in *Chlorella pyrenoidosa*, into two major Chl *a* components (Chl *a* 670 and Chl *a* 680), and one Chl *b* component (Chl *b* 650). Minor components of Chl *a* are supposed to exist at 690–700 nm. (C. Cederstrand, E. Rabinowitch, and Govindjee, 1966.) This figure is plotted on a wavenumber (reciprocal wavelength) scale.

scattering and, more importantly, there is "selective scattering" around the absorption band, found in 1956 by P. Latimer in our laboratory. Scattering increases the apparent absorption, if measured in an ordinary spectrophotometer, and can totally distort the absorption spectra.

3. In a scattering suspension, light that is less strongly absorbed has a larger effective path length than the more strongly absorbed light (this is known as the *detour effect*).

4. A mutual shading of the pigment molecules takes place in densely colored particles, such as the chloroplasts, while some light passes the suspension without hitting any particle at all (this has been called the *sieve effect*).

5. Fluorescence contributes to the transmitted light, particularly significantly when the absorption is strong and the fluorescence yield is high.

Two methods have been used to remedy some of these difficulties: (a) An opal glass can be placed between the sample and the detector, so that most of the forward-scattered light is collected (much of the scattered light in a suspension of large particles leaves the suspension in the forward direction). (b) An integrating sphere (Ulbricht sphere) can be used for collecting all (or, at least, almost all) the scattered energy. However, neither of the two methods eliminates the sieve or the detour effect.

C. S. French at the Carnegie Institution of Washington at Stanford, California, has constructed a "derivative" spectrophotometer in which the first derivative of the absorbance is plotted automatically as function of wavelength. This procedure accentuates the complex structure of a band, since every inflection in the band envelope appears either as crossing of the abscissa or as a peak. The "derivative absorption spectra" of various algae, obtained in this way, were analyzed by French and co-workers into three components (to which a certain standard shape, that of a so-called Gaussian error curve, has been ascribed for the purpose of analysis). They were identified as Chl *a* 672, Chl *a* 683, and Chl *a* 694. In our laboratory, Carl Cederstrand has measured the absorption spectra of algae in an integrating dodecahedron with one photocell on each of its 12 faces, and analyzed the results with the help of a computer. The red band of chlorophyll *a* was found to be essentially double with its envelope covering two neighboring bands (Fig. 9.7), like a couple huddling under a single raincoat (a couple with a child, if

the chlorophyll *b* band is included). One band was located at 668 nm (French's "Chl *a* 672"), the other at 683 nm (French's "Chl *a* 683")—again assuming a Gaussian shape of the component bands. This assumption is not completely arbitrary, since a Gaussian curve was found to match very closely the red band of Chl *a* in solution. (Deviations from the Gaussian curve are, however, visible on both ends of the band in Fig. 9.7.)

Later, we found that elimination of the "sieve effect" by breaking Chlorella cells with ultrasonic waves (making the particles small enough to reduce shading to insignificance), does not affect the qualitative results of the analysis; but, as expected, it moves the peaks of the two chlorophyll *a* components closer together—to 670 and 680 nm, respectively. The half band-width of the Chl *a* 670 and Chl *a* 680 bands was found to be about 17 nm, that is very similar to that of the Chl *a* band in ether.

Cederstrand's analysis gave no evidence for the existence of a third long-wave component (such as French's Chl *a* 694). The computer, asked to explain in the simplest possible way the shape of the red band envelope in terms of Gaussian components, answered that three components (Chl *b* and two forms of chlorophyll *a*) provide as good an approximation as can be reasonably expected. Because of deviations from Gaussian shape in the wings in the Chl *a* band in vitro, it seemed unjustifiable to postulate additional components in order to obtain a better fit in the long-wave wing of the band in vivo.

However, quite apart from this analysis, there is considerable evidence for the existence of one (or two) minor absorption bands of Chl *a* in the 690–700 nm region. One is the position and the shape of the "red drop" in the action spectra of Chl *a* fluorescence and of photosynthesis (see Chapters 13 and 15). It indicates a minor Chl *a* form, not contributing to fluorescence, and contributing only partially to photosynthesis, with an absorption band in the region of 695 nm. Another is the "difference spectrum" obtained by subtraction of the spectrum of Chlorella sonicates obtained at neutral pH and under anaerobic conditions, from that of sonicates obtained at acid pH under aerobic conditions. This spectrum, too, shows a band at 693 nm, suggesting that Chl *a* 693 is present in a small amount in living cells, and is particularly easily destroyed by ultrasonic irradiation in an aerobic acid medium.

If one introduces a 693 nm band into Cederstrand's analysis, the result

is a slight shift of the main long-wave component, Chl *a* 680, towards the shorter waves, and a change in the ratios of the two peaks in favor of Chl *a* 670.

Additional minor Chl *a* components (0.3% or less of total chlorophyll), with maxima at 700 nm (P700) and at 682–690 (P690) have been suggested on the basis of difference spectroscopy (Chapter 14). Their concentrations are too small for them to be noticeable in the analysis of the absorption spectrum.

Spectroscopic evidence for the complexity of chlorophyll *a* in vivo is supported by chemical evidence, presented particularly by T. M. Godnev and A. A. Shlyk in Minsk (Byelorussia). They noted that during gradual extraction of chlorophyll from leaves, the absorption spectrum changed. J. H. C. Smith and co-workers, at the Carnegie Institution in Stanford, have similarly demonstrated that during the greening of leaves, the red absorption band undergoes shifts. In these experiments, leaves were permitted to form in the dark. They are then colorless or "etiolated." When exposed to light, they rapidly become green. Altogether, it seems as if two or three spectroscopically different forms of chlorophyll *a* are formed at different times, and are extracted with different ease.

Fractionation of these two (or three) Chl *a* forms by breaking the cells mechanically and solubilizing the pigment complexes selectively by means of detergents such as desoxycholate or digitonin, or by extraction with solvents of varying polarity, has been attempted in several laboratories. These observations are relevant to the question of whether some components of Chl *a* are associated with one or the other of the two photochemical reactions postulated in photosynthesis and with the two "pigment systems" supposed to sensitize them. We shall discuss this point in Chapter 16.