

Chapter 8

Structure and Composition of the Photosynthetic Apparatus

CHLOROPLASTS, GRANA, LAMELLAE

It was said before that photosynthesis requires a certain subcellular structure. Probably, it is needed to direct the reaction sequence, and to prevent unstable intermediates from getting off their tracks.

Not long ago, chemical processes inside a living cell were considered as reactions in a homogenous solution confined in a bag. True, certain structures, granules or fibrils, had been early observed in the cell under the light microscope, but these were skeptically considered as artifacts resulting from slicing or staining. Subcellular particles clearly visible under the microscope, such as cell nuclei or chloroplasts, were considered as "bags within bags."

The invention of the electron microscope, with a much stronger resolving power (roughly speaking, 10^{-7} instead of 10^{-5} cm), brought unequivocal proof that the nuclei, the chloroplasts, and other "organelles," imbedded in the cytoplasm, as well as the cytoplasm itself, possess an internal structure undoubtedly significant for their metabolic functions.

The chloroplasts of the higher plants are shown in Fig. 8.1 as they



FIG. 8.1 Chloroplasts (c) in leaf cells (optical micrograph). (Courtesy of D. Paolillo.)

appear under the light microscope. They are more or less ellipsoidal bodies, $5\text{--}10\mu$ in diameter, or about one fifth or one tenth the size of the cell (one μ is a thousandth of a millimeter, or 10^{-4} cm). In algae, the shapes of chloroplasts are more varied. In elongated algal fronds, they may be long, twisted bands. In some approximately spherical cell, they are single star-shaped bodies. The unicellular green alga *Chlorella*, often mentioned in this text, contains a single chloroplast shaped like a bell, adhering to the inside of the cell wall (Fig. 8.2).

The first structures discovered within chloroplasts by means of the



FIG. 8.2 Electron micrograph of *Chlorella* showing the cup-shaped chloroplast, magnification 24,000 \times . (Courtesy of T. Bisalputra, University of British Columbia, Canada.)

electron microscope were the so-called *grana*, flat tablets about 0.5μ in diameter and about 0.3μ thick. Several dozen grana may be found in a single chloroplast (Fig. 8.3).

Later, it became clear that in *intact* chloroplasts, the grana are parts of a system of *lamellae*, stretching through the chloroplast (Fig. 8.4).

Some chloroplasts seem to contain only lamellae and no grana at all, or only vague outlines of them, as in Fig. 8.5.

Figure 8.6 shows a slice through a leaf of corn (*Zea mays*), in which the chloroplasts in the leaf parenchyma are clearly granular, while those in the vascular tissue show only lamellae. It seems that grana are approximately cylindrical regions formed by stacking of electron-dense sections of the lamellae.

The lamellar structure is found not only in the ellipsoidal chloroplasts of the higher plants, but also in algal chloroplasts of other shapes, although in the latter, the lamellae may be involuted rather than plane-

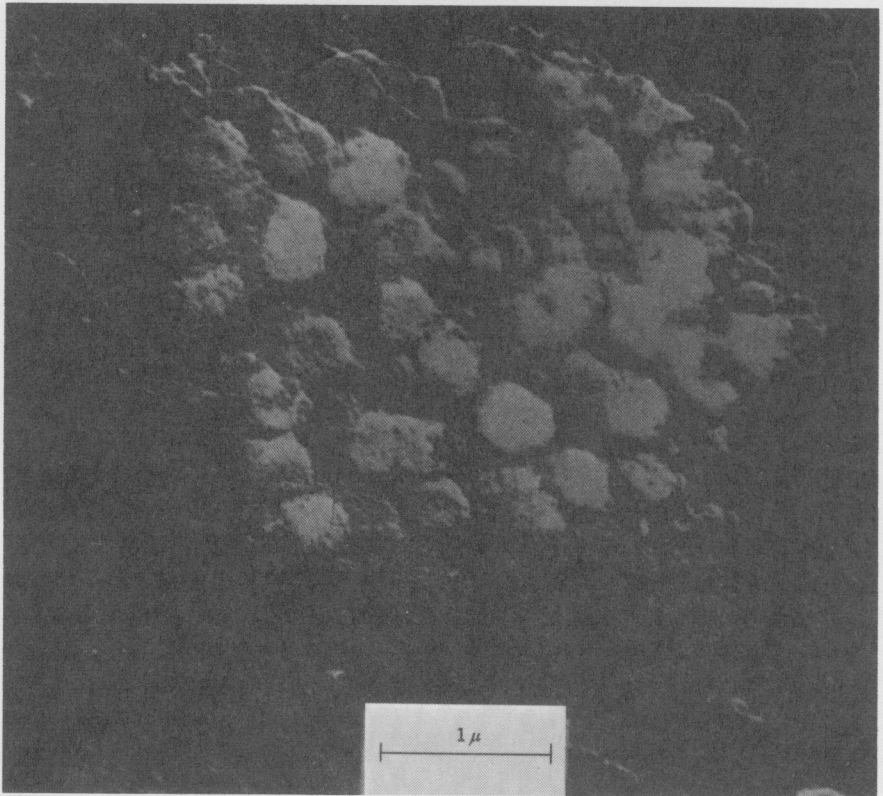


FIG. 8.3 Grana in a collapsed chloroplast of maize (*Zea mays*). (A. E. Vatter, 1952.)

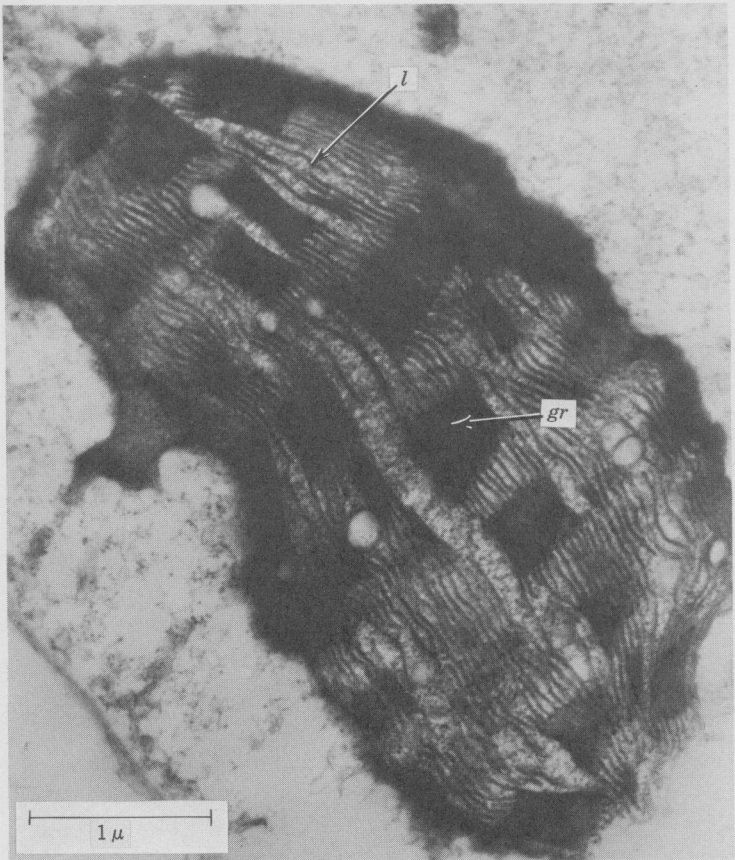


FIG. 8.4 Lamellae inside and outside the grana in maize (*Zea mays*). (*gr*, grana; *l*, lamellae.) (A. E. Vatter, 1955).

parallel (Figs. 8.2 and 8.7). The most primitive of plants, the blue-green algae (*Cyanophyceae*), contain no separate chloroplasts at all; but they do contain lamellae, extending through the cytoplasm.

The lamellae are a special case of two-dimensional cellular structures called membranes. Biological membranes generally consist of two superimposed layers; such a double layer is often referred to as the "unit membrane." Each lamella in chloroplasts may contain *two* such unit membranes, one on top of the other. W. Menke (Köln, Germany) has observed in photosynthetic bacteria more or less spherical "sacks" with



FIG. 8.5 Chloroplast from a light green part of the inner leaf of a young shoot of *Aspidistra elatior*. Lamellae without grana. (H. Leyon, 1954.)

double-layered walls, which he called *thylakoids* (Fig. 8.8). (These thylakoids are *not* "rooms without doors and windows"; extensive interconnections exist between them). The lamellae observed in higher plants may be flattened thylakoids, or assemblies of such thylakoids, fused together into flat "double pancakes."

With improved techniques of electron microscopy, more details were observed in chloroplast pictures, and more elaborate structures were suggested for the lamellae. We cannot enter here into the description of these details, particularly since most of them still remain speculative.¹ Instead, we return to the "photosynthetic units," whose existence has been derived in Chapter 6 from the kinetics of photosynthesis.

¹See articles by W. Menke, and by R. B. Park and D. Branton, and by T. E. Weier, C. R. Stocking, and L. K. Shumway, in *Energy Conversion by the Photosynthetic Apparatus*, Brookhaven National Laboratory, January, 1967.

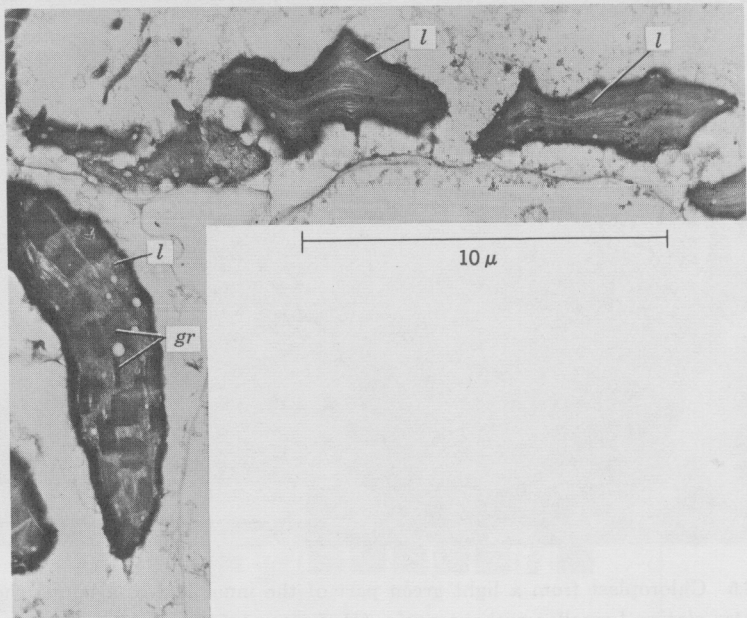


FIG. 8.6 *Zea mays* leaf section, showing granular chloroplasts in the leaf parenchyma (left) and nongranular chloroplasts in the vascular bundle (top right). (*gr.* grana; *l.* lamellae.) (A. E. Vatter, 1955.)



FIG. 8.7 Lamellae (*l*) in red alga *Porphyridium cruentum* (Marcia Brody and A. E. Vatter, 1958.)

PHOTOSYNTHETIC UNITS

In Chapter 6, it was noted that photosynthesis requires a large number of light-absorbing pigment molecules to supply excitation energy to a much smaller number of enzymatic "centers." Experiments were described there that suggested cooperation of about 300 chlorophyll *a* molecules with a single enzymatic center. These assemblies were called "photosynthetic units." Certain granular structures in chloroplast lamellae, observed under the electron microscope can be tentatively identified with these kinetic units. A "cobblestone pavement" picture of chloroplast lamellae was first observed in 1952 by E. Steinmann in Zurich. It appeared much clearer in pictures obtained by R. B. Park and J. Biggins at Berkeley, California, in 1961 (Fig. 8.9). The structure is particularly

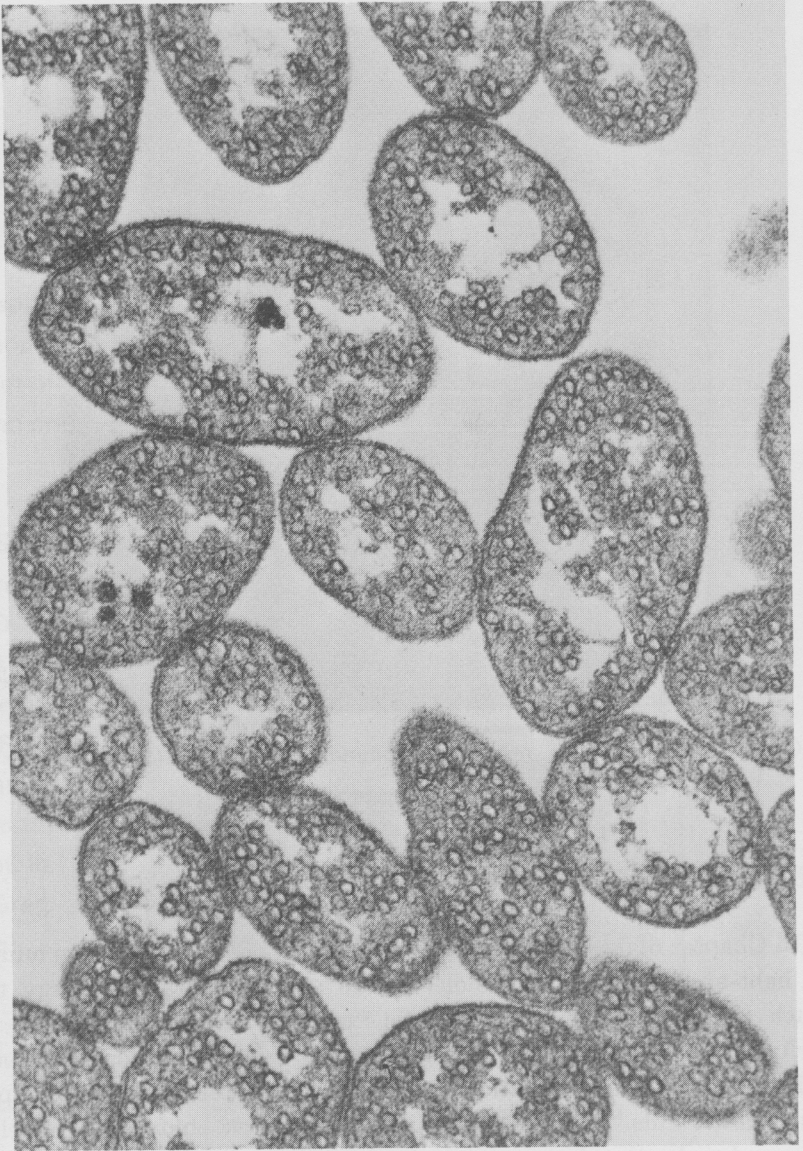


FIG. 8.8 Vesicle-like thylakoids ($\times 35,000$) in *Rhodospseudomonas spheroides*. (W. Menke, 1966.)

regular in some areas where the membrane, apparently enveloping the lamella like a pillow-case envelopes a pillow, has been torn away. Park and co-workers dubbed the granular units “quantasomes,” in the belief that they are the sites of the primary photochemical process—they called it “quantum conversion”—in photosynthesis. The size of the quantasomes is about $18 \times 16 \times 10.0$ nm; their upper surface is thus about 300 nm^2 . Chlorophyll molecules in artificial monolayers, stacked obliquely, like books on a half-filled shelf, are known to occupy about 1 nm^2 each. (When put flat on their “faces,” they cover about 2.4 nm^2 each.) Thus,

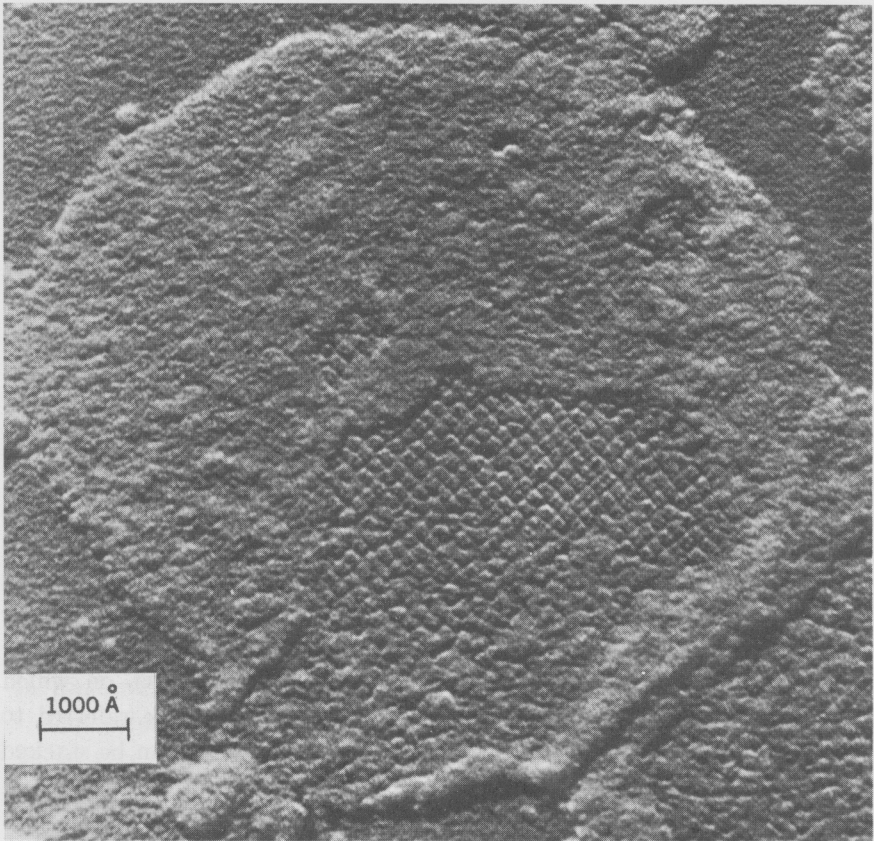


FIG. 8.9 “Quantasomes” on a chloroplast lamella. (R. B. Park and J. Biggins, 1964.) ($1000\text{Å} = 1000 \text{ nm}$.)

a quantasome may offer just enough surface space to the about 300 chlorophyll molecules postulated to be present in a photosynthetic unit.

However, some recent experiments by E. N. Moudrianakis, (at Johns Hopkins University) suggested that "quantasomes" can be removed from the lamellae, like mushrooms cut off from a bed, and that the remaining lamellae can still reduce certain Hill oxidants in light. This argues against identification of "quantasomes" with complete photosynthetic units. However, it is not certain that Park and Moudrianakis had been looking at the same particles.

PROTEINS AND LIPOIDS

If one separates, by maceration and centrifugation, chloroplasts (or their fragments) from the rest of the cell material and analyzes them, one finds that, in contrast to the cytoplasm in which they were suspended (which is almost pure protein), chloroplasts contain 30-40 percent of nonproteidic material. This is classified as "lipoid" (fat-like), because it is insoluble in water but soluble in typical fat-solvents (such as alcohol and ether). Some of the chloroplast lipoids contain phosphate residues (phospholipids); others contain carbohydrates (glucolipids).

Lipoids are widespread in nature, and are known to perform two main biological functions. One is *energy storage*, associated with the high energy content of fats (that is, their high reduction level; see Chapter 5). The other is *selective permeability*. Lipoids are found in all membranes: the frog's skin, the membrane surrounding red blood corpuscles, the sheaths that enclose nerve fibers, etc. All such membranes contain 30-40 percent lipoid material, the rest being fibroid (threadlike, as contrasted to globular) proteins. A general picture has developed of membranes being built like plastic tablecloths, with a fibrous network underlying a plastic layer. The proteins provide the fibrous base, on which the amorphous lipoid layer is spread. This is the structure referred to above as the "unit membrane." Many such double layers can be stacked on top of each other, for example in nerve sheaths, chloroplasts, visual cones, and other cell organelles. All of them consist of alternating proteidic and lipoidic layers. In chloroplasts, the lamellae are closely packed inside the grana; outside the grana, they are separated by so-called "stroma" probably a pure proteidic medium (Fig. 8.4).

The function of membranes, when they are found surrounding a cell organ, a whole cell, or the organism as a whole, is to maintain inside a chemical composition different from that prevailing outside, while permitting a certain material exchange without which no life is possible. These membranes have so-called selective permeability, letting some molecules through, but preventing the passage of others. It is not enough to keep, let us say, large molecules inside (or outside) while letting smaller ones circulate freely, as could be done by a molecular sieve with holes of appropriate size. A distinction is made by many cell membranes between different simple molecules or ions, such as those of sodium and potassium. The membrane must thus contain a mechanism for selecting admissible from nonadmissible ions and molecules. More than that, it often has a mechanism for *active diffusion*, that is, for pumping certain molecules or ions in from the outside (and others, out from the inside) even *against a concentration gradient*, from lower to higher concentrations. (Natural or passive diffusion always goes in the opposite direction, as required by the entropy principle.)

The selective permeability of cell membranes is associated, at least in part, with the properties of thin lipid layers. In some simple cases, the relevant factor is simply the solubility of various compounds in the lipoids. Those that are easily soluble penetrate the membrane easier than those that are practically insoluble. This is, however, not the whole story. To explain such phenomena as allowing the passage of potassium and barring the passage of sodium (or vice versa), special mechanisms have to be imagined. Since any motion against the gradient of concentration consumes free energy, it can occur only by coupling it with some free energy-supplying process, such as respiration (probably via high energy phosphate, ATP, as energy carrier). The details of the coupling mechanism remain to be elucidated, although some promising suggestions have been made in recent years, particularly by P. Mitchell in England.

The prime function of the protein layer in a membrane often may be to give it mechanical strength, because protein molecules consist of extended chainlike molecules, in which the links are strong chemical bonds and cannot be easily disrupted. These chains can form very stable mats, containing pores through which diffusion of even relatively large molecules can proceed. However, some proteins in the membrane must have a more active, catalytic function.

In chloroplasts, the stacked membranes may have another function. They offer support for a display of enzymes, permitting easy access of substrate molecules, directed passage of intermediates from one enzyme to another, and easy removal of the ultimate products. They provide, as it were, long metabolic conveyor belts. (The same picture applies to mitochondria, the site of several energy-releasing oxidation-reduction reaction steps in respiration.) A third function of membranes can be envisaged in chloroplasts (and, perhaps, also in visual rods): Absorbed light quanta must be efficiently utilized in them—in photosynthesis for photochemical energy storage and in vision for the initiation of electric signals in the optic nerve. Efficient propagation of *energy*, in addition to efficient transfer of *chemical entities*, may be needed to permit excitation to reach the site of its utilization.

The most important function of proteins in the living organism is to serve as catalysts in metabolic processes. Enzymes, the all-important biological catalysts, are such catalytic proteins; often they carry certain relatively small active (“prosthetic”) groups directly involved in their catalytic action. All enzymes needed for the conversion of CO₂ to carbohydrates are located in the chloroplasts; some other enzymes (such as, *catalase* and *peroxidase*) have been found in them, too.

Certain colored proteins, such as the *iron*-porphyrin-containing *cytochromes*, have been found in chloroplasts and appear to serve as catalysts in photosynthesis. Like hemoglobin, they contain an iron atom in the center of a porphyrin molecule. (Other cytochromes play an important role in respiration in animals and plants.) More recently, a *copper*-containing, blue protein was found in chloroplasts and called *plastocyanin*; it, too, is supposed to have a catalytic function in photosynthesis. We shall return to these catalysts in Chapter 14. A *manganese*-containing enzyme has been implicated in photosynthesis, but no manganese-bearing protein has as yet been isolated from chloroplasts.

All chloroplast pigments seem to be attached to proteins. In the case of the (red and blue) phycobilins, the pigments can be easily and completely extracted, together with their carrier protein, into an aqueous medium.

Chlorophyll-protein complexes have been isolated from green bacteria, blue-green algae, and green plants; but not more than a small fraction of total chlorophyll was obtained in this form.

NUCLEOTIDES AND QUINONES

Another kind of nitrogen-containing basic life constituents, in addition to the long-known proteins, are the so-called nucleotides. In *nucleic acids*—the carriers of genetic information in the chromosomes—four types of nucleotides alternate in long chains, like letters in a script. Other nucleotides serve as catalysts in metabolic reactions. Chloroplasts contain two kinds of the latter—nicotinamide adenine dinucleotide (NAD^+), and nicotinamide adenine dinucleotide phosphate (NADP^+).

Among the “quinonoid” compounds present in chloroplasts, that is, compounds with a structure similar to that of simple quinones (see Chapter 5), and capable of reversible reduction to hydroquinone-like compounds, are the so-called *plastoquinones*.

We shall see in Chapters 14 and 17 that several above-mentioned compounds—cytochromes, plastoquinone, plastocyanin, and pyridine nucleotides—are assumed to act as intermediates in the path of hydrogen atoms (or electrons) from H_2O to CO_2 (see Figs. 5.4 and 14.4). They lose and acquire electrons reversibly, like members of a bucket brigade pass pails of water.

THE PIGMENTS: THEIR LOCATION IN THE CHLOROPLASTS

The chloroplast pigments are the most important specific components of the chloroplasts. They absorb light and initiate the chains of enzymatic reactions that lead to the conversion of H_2O and CO_2 into carbohydrates and oxygen. They are present in large amounts (up to 5% of the dry cell material by weight!). This is needed for photosynthesis to proceed in the practically available light fast enough to support the growth of the plant. (Because of the endergonic character of photosynthesis, no light-initiated chain reactions are possible!)

Because of this importance, the pigments will be discussed in a separate chapter (Chapter 9). Here, we shall only deal with their location in the chloroplasts. Unfortunately, electron micrographs tell us nothing

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about the location of pigments. Electrons do not distinguish between colored and colorless material, but only between materials of different density of the electronic cloud, that is, between lighter and heavier atoms. (Higher atomic number means a higher charge of the nucleus, and thus also a denser crowd of electrons around it!) The sharp relief in Fig. 8.3 was obtained by "staining" the preparation, by depositing on it heavy metal atoms from an atomic beam (created by heating a metal grain in vacuum). In other electron-micrographs, the contrast is achieved by means of heavy osmium atoms precipitated by chemical reduction of osmium tetroxide (osmic acid, OsO_4). The osmium atoms are precipitated preferentially, in (or on) layers of a stronger reducing power. It is not quite certain yet what these layers are, but it does seem likely that the darker layers (see Fig. 8.4) are more lipoidic in nature, and the lighter ones, more proteidic.

In any case, even the best electron micrographs tell us nothing about the location, in the layered structure, of the molecules of chlorophyll or of other plant pigments.

A plausible picture of this location can be based on the consideration of the structure of the chlorophyll molecules, and its likely behavior in a system of alternating proteidic and lipoidic layers. These layers are hydrophilic and hydrophobic, respectively. Water molecules are electric dipoles, with a positive charge on the hydrogen side and a negative charge on the oxygen side of the molecules. They are, therefore, attracted by free ions, which, in contact with water, surround themselves by a halo of water molecules (Fig. 8.10*a*), and also by ionized or polarized spots in large molecules. In the latter case, water dipoles crowd around the polar spot like wasps around a drop of honey (Fig. 8.10*b*). Protein molecules contain many charged spots, both negative (acidic) and positive (basic). Therefore, they are more or less strongly hydrophilic (water-attracting). Many protein molecules are, in fact, soluble in water; that is, they attract so many water molecules that the latter bodily lift and carry them into the aqueous medium. A proteidic layer is, therefore, hydrophilic. Lipids (fats), on the other hand, are hydrophobic (water-repellent), because they contain no polar groups and exercise only general "van der Waals" attraction forces on all neighboring molecules. We cannot discuss here the nature of the van der Waals forces. (F. London has interpreted them as being due to the fact that even uncharged molecules consist of moving positive and negative particles;

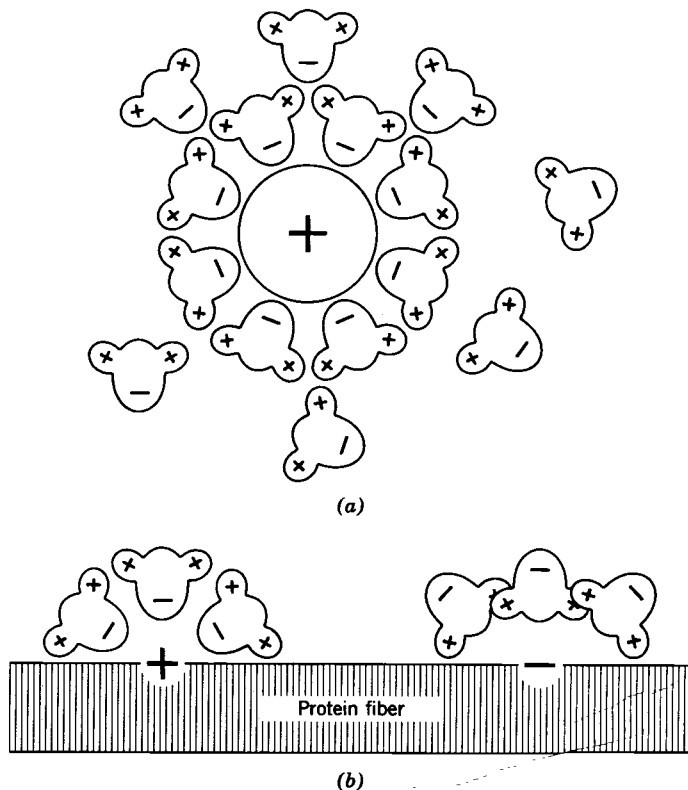


FIG. 8.10 (a) Water dipoles around an ion. (b) Water dipoles around charged spots on a protein molecule.

when two such molecules approach each other, these particles are displaced and the originally nonpolar molecules become “dipoles”—and dipoles attract each other.) In competition between polar water molecules and nonpolar organic molecules for positions close to other nonpolar organic molecules, the second ones win out, the water molecules are squeezed out. It is not that such “hydrophobic” molecules “dislike” water, but they like organic molecules more!

Phospholipids are lipoids that contain phosphoric acid anions; these *are* polar, so that water molecules are attracted to them. In other words, hydrophobic molecules may contain hydrophilic spots. The overall behavior of such molecules is the balance of their hydrophobic and hydro-

philic properties. Thus, *soaps* and *detergents* attach themselves to fat molecules by their hydrophobic parts, and carry them into water by means of their hydrophilic parts.

When a molecule that contains both hydrophilic and hydrophobic parts is brought into a structure consisting of alternating hydrophilic and hydrophobic layers, one expects it to find a most stable position at the protein-lipoid *interface*, where it can satisfy both its attraction to water and its affinity for organic molecules.

We know (see Chapter 9) that the chlorophyll molecule is built like a tadpole, with a roughly square head (the porphyrin ring) and a long phytol tail. The head is polar because it contains the magnesium atom, which tends to acquire a positive charge, making the rest of the molecule negative, and this polarity causes an affinity to water. The phytol tail, on the other hand, is nonpolar and therefore hydrophobic. Chlorophyll molecules should therefore accumulate on lipid-protein interfaces, with their flexible phytol tails dipping into the lipid layers, and the rigid porphyrin head attracted to the aqueous layer. Chlorophyll may thus form a one-molecule thick layer on the interface between the lipid and the protein lamellae in chloroplasts (Fig. 8.11). Optical studies of such properties of chloroplasts as birefringence and dichroism support the idea that chlorophyll molecules form separate layers between the proteidic and the lipoidic lamellae.

Preparing artificial pigment monolayers by evaporating a drop of chlorophyll solution in petroleum ether spread on the surface of water, can lead to two types of layers, crystalline and amorphous. Crystalline chlo-

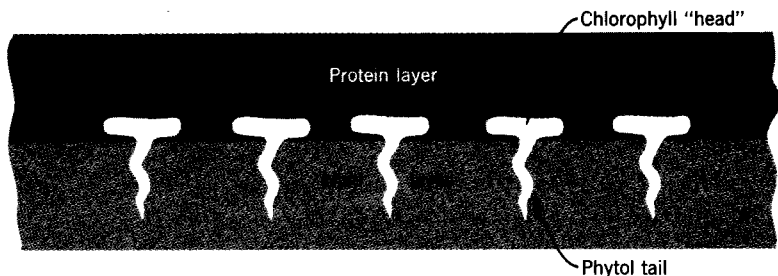


FIG. 8.11 Chlorophyll molecules on the interface between lipid and protein lamellae.

rophyll layers are characterized by greater surface density, and by a considerable shift in the position of the red absorption band—from 660–670 nm in solution, to 730–740 nm, probably caused by molecular interactions in a closely packed regular two-dimensional lattice (see absorption bands a and d in Fig. 8.12). Noncrystalline chlorophyll monolayers are less dense, and have absorption bands only slightly shifted from their position in solution (to 670–680 nm; see Fig. 8.12*b*). The absorption spectra of pigments in live cells show no evidence of crystalline structure; the red absorption band of chlorophyll *a* in the living cells is located at 670–680 nm (Fig. 8.12*c*). Thus, if chlorophyll monolayers *are* present in chloroplasts, they must be of amorphous, rather than crystalline kind. Some evidence has been found in recent years that a small fraction of chlorophyll *a* in vivo may have a more orderly arrangement than the bulk of it. These may be the molecules

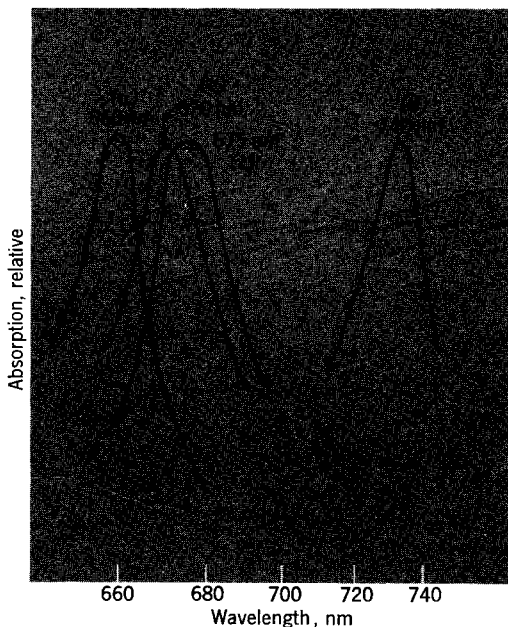


FIG. 8.12 “Tops” of the red absorption band of chlorophyll *a*, in true solution (a); in colloidal solution or amorphous monolayer (b); in living cell (c); and in a crystalline monolayer (d). Note greater width of the band in (c).

most closely associated with the "reaction centers" in the photosynthetic units.

The alternation of lipoidic and proteidic layers on two sides of chlorophyll layers may permit the two primary reaction products, the strong oxidant and the strong reductant, to escape from each other, and thus prevent a back reaction between them.

We mentioned before the existence of "photosynthetic units" often containing about 300 Chl *a* molecules in green plants and about 50 bacteriochlorophyll molecules in bacteria. How can the existence of such units be reconciled with the concept of extended monomolecular pigment

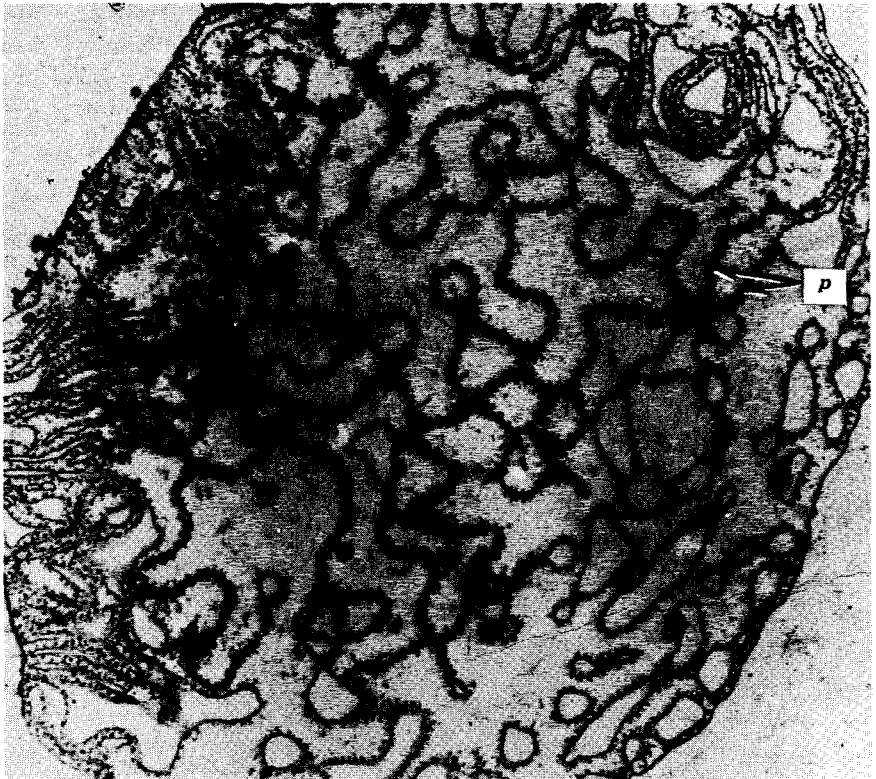


FIG. 8.13 *Phycobilisomes* (*p*), in chloroplast of *Porphyridium cruentum*. (E. Gantt and S. F. Conti, 1966.)

layers? Perhaps, the units are "cobblestones" in the "pavement" of the lamellae. This point remains to be elucidated.

Even if we assume that chlorophyll molecules are arranged in monomolecular layers on protein-lipoid interfaces, many questions remain unanswered, such as: Are chlorophyll *a* and chlorophyll *b* mixed indiscriminately in this green coat? Where are the carotenoids? Where are the phycobilins? Recent electron micrographs by E. Gantt and S. F. Conti at the Dartmouth Medical School have shown small phycobilin-containing granules, approximately 35 nm in diameter, attached to the chloroplast lamellae in the red alga *Porphyridium* (Fig. 8.13). The name *phycobilisomes* has been suggested for them. Here again, the problem arises of relation between such three-dimensional structures and the postulated monomolecular pigment layers.

Detailed models of the arrangement of the pigments and other components in the chloroplast lamellae have been constructed by many biologists, but they remain speculative.

Finally, the concept (see Chapters 13–17) of two separate photochemical systems (and thus, presumably, also of two types of photosynthetic units, of equal or different size) being involved in photosynthesis, remains to be reconciled with the pictures presented in this chapter. We shall deal in Chapter 16 with experiments suggesting that units of the two types can be separated, at least partially, by certain fractionation procedures.