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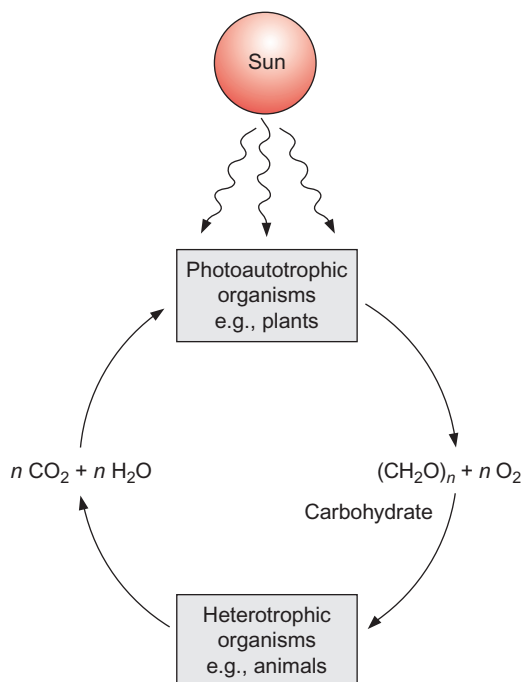
The use of energy from sunlight by photosynthesis is the basis of life on earth

Plants and cyanobacteria capture the light of the sun and utilize its energy to synthesize organic compounds from inorganic substances such as CO_2 , nitrate, and sulfate to synthesize their cellular material; they are photoautotrophic. In photosynthesis photon energy splits water into oxygen and hydrogen, the latter bound as NADPH. This process, termed the *light reaction*, takes place in the photosynthetic reaction centers embedded in membranes. It involves the transport of electrons, which is coupled to the synthesis of ATP. NADPH and ATP are consumed in a so-called dark reaction to synthesize carbohydrates from CO_2 (Fig. 2.1). The photosynthesis of plants and cyanobacteria created the biomass on earth, including the deposits of fossil fuels and atmospheric oxygen. Animals are dependent on the supply of carbohydrates and other organic compounds as food; they are heterotrophic. They generate the energy required for their life processes by oxidizing the biomass, which has first been produced by plants. When oxygen is consumed, CO_2 is formed. Thus light energy captured by plants is the source of energy for the life processes of animals.

2.1 How did photosynthesis start?

Measurements of the distribution of radioisotopes led to the conclusion that the earth was formed about 4.6 billion years ago. The earliest indicators of life on earth are fossils of bacteria-like structures, estimated to be 3.5 billion years old. There was no oxygen in the atmosphere when life on

Figure 2.1 Life on earth involves a CO₂ cycle.



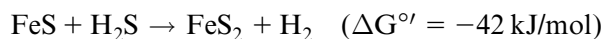
earth commenced. This is concluded from the fact that in very early sediment rocks iron is present as Fe^{2+} . Mineral iron is oxidized to Fe^{3+} in the presence of oxygen. According to our present knowledge, the earth's atmosphere initially contained components such as carbon dioxide, molecular hydrogen, methane, ammonia, prussic acid, and water.

In 1922 the Russian scientist Alexander Oparin presented the interesting hypothesis that organic compounds were formed spontaneously in the early atmosphere by the input of energy (e.g., in the form of ultraviolet radiation (there was no protective ozone layer), electrical discharges (lightning), or volcanic heat). It was further postulated that these organic compounds accumulated in ancient seas and became the constituents of early forms of life. In 1953 the American scientists Stanley Miller and Harold Urey substantiated this hypothesis by simulating the postulated **prebiotic synthesis** of organic substances. They exposed a gaseous mixture of components present in the early atmosphere, consisting of H_2O , CH_4 , NH_3 and H_2 to electrical discharges for about a week at 80°C . Amino acids (such as glycine and alanine) and other carboxylic acids (such as formic, acetic, lactic, and succinic acid) were found in the condensate of this experiment. Other investigators added substances such as CO_2 , HCN , and formaldehyde to the gaseous mixture, and these experiments showed that many components

of living cells (e.g., carbohydrates, fatty acids, tetrapyrroles, and the nucleobases adenine, guanine, cytosine, and uracil) were formed spontaneously by exposing a postulated early atmosphere to electric or thermal energy.

It is assumed that the organic substances formed by the abiotic processes accumulated in the ancient seas, lakes, and pools over a long period of time prior to the emergence of life on earth. There was no oxygen to oxidize the compounds that had accumulated and no bacteria or other organisms to degrade them. Alexander Oparin speculated that a “**primordial**” **soup** was formed in this way, providing the building material for the origin of life. Since oxygen was not yet present, the first organisms must have been **anaerobes**.

It is widely assumed now that early organisms on this planet generated the energy for their subsistence by **chemolithotrophic metabolism**, for example, by the reaction:



It seems likely that already at a very early stage of evolution the catalysis of this reaction was coupled to the generation of a proton motive force (section 4.1) across the cellular membrane, yielding the energy for the synthesis of ATP by a primitive ATP synthase (section 4.3). **Archaeobacteria**, which are able to live anaerobically under extreme environmental conditions (e.g., near hot springs in the deep sea), and which are regarded as the closest relatives of the earliest organisms on earth, are able to produce ATP via the preceding reaction. It was probably a breakthrough for the propagation of life on earth when organisms evolved that were able to utilize the energy of the sun as a source for biomolecule synthesis, which occurred at a very early stage in evolution. The now widely distributed **purple bacteria** and **green sulfur bacteria** may be regarded as relics from an early period in the evolution of photosynthesis.

Prior to the description of photosynthesis in Chapter 3, the present chapter will discuss how plants capture sunlight and how the light energy is conducted into the photosynthesis apparatus.

2.2 Pigments capture energy from sunlight

The energy content of light depends on its wavelength

In Berlin at the beginning of the twentieth century Max Planck and Albert Einstein, two Nobel Prize winners, carried out the epoch-making studies proving

that light has a dual nature. It can be regarded as an electromagnetic wave as well as an emission of particles, which are termed **light quanta** or **photons**.

The energy of the photon is proportional to its frequency ν :

$$E = h \cdot \nu = h \cdot \frac{c}{\lambda} \quad (2.1)$$

where h is the Planck constant ($6.6 \cdot 10^{-34}$ J s) and c the velocity of the light ($3 \cdot 10^8$ m s⁻¹). λ is the wavelength of light.

The mole (abbreviated to mol) is used as a chemical measure for the amount of molecules and the amount of photons corresponding to $6 \cdot 10^{23}$ molecules or photons (Avogadro number N_A). The energy of one mol photons amounts to:

$$E = h \cdot \frac{c}{\lambda} \cdot N_A \quad (2.2)$$

In order to utilize the energy of a photon in a thermodynamic sense, this energy must be at least as high as the Gibbs free energy of the photochemical reaction involved. (In fact much energy is lost during energy conversion (section 3.4), with the consequence that the energy of the photon must be higher than the Gibbs free energy of the corresponding reaction.) We can equate the Gibbs free energy ΔG with the energy of the absorbed light:

$$\Delta G = E = h \cdot \frac{c}{\lambda} \cdot N_A \quad (2.3)$$

The introduction of numerical values of the constants h , c , and N_A yields:

$$\Delta G = 6.6 \cdot 10^{-34} \cdot (\text{J} \cdot \text{s}) \cdot \frac{3 \cdot 10^8 (\text{m})}{(\text{s})} \cdot \frac{1}{\lambda (\text{m})} \cdot \frac{6 \cdot 10^{23}}{(\text{mol})} \quad (2.4)$$

$$\Delta G = \frac{119000}{\lambda (\text{nm})} \quad [\text{kJ/mol photons}] \quad (2.5)$$

It is often useful to state the electrical potential (ΔE) of the irradiation instead of energy when comparing photosynthetic reactions with redox reactions, which will be discussed in Chapter 3:

$$\Delta E = -\frac{\Delta G}{F} \quad (2.6)$$

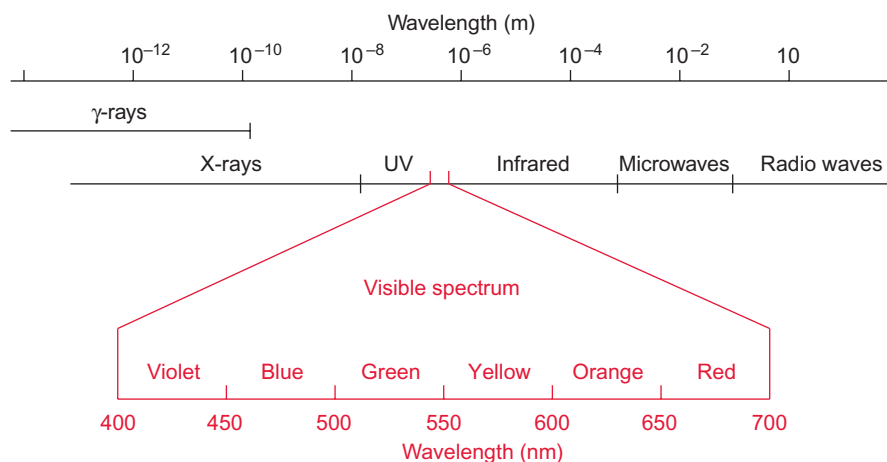


Figure 2.2 Spectrum of the electromagnetic radiation. The enlargement in red illustrates the visible spectrum.

where F = number of charges per mol = $96,485 \text{ Amp} \cdot \text{s} \cdot \text{mol}^{-1}$. The introduction of this value yields:

$$\Delta E = -\frac{N_A \cdot h \cdot c}{F \cdot \lambda(\text{nm})} = \frac{1231}{\lambda(\text{nm})} \text{ [Volt]} \quad (2.7)$$

The human eye perceives only the small range between about 400 and 700 nm of the broad spectrum of electromagnetic waves (Fig. 2.2). The light in this range, where the intensity of solar radiation is especially high, is utilized in plant photosynthesis. Bacterial photosynthesis, however, is able to utilize light in the infrared range.

According to equation 2.3 the energy of irradiated light is inversely proportional to the wavelength. Table 2.1 shows the light energy per mol photons for light of different colors. Consequently, violet light has an energy of about 300 kJ/mol photons. Dark blue light, with the highest wavelength (700 nm) that can still be utilized by plant photosynthesis, contains 170 kJ/mol photons. This is only about half the energy content of violet light.

Chlorophyll is the main photosynthetic pigment

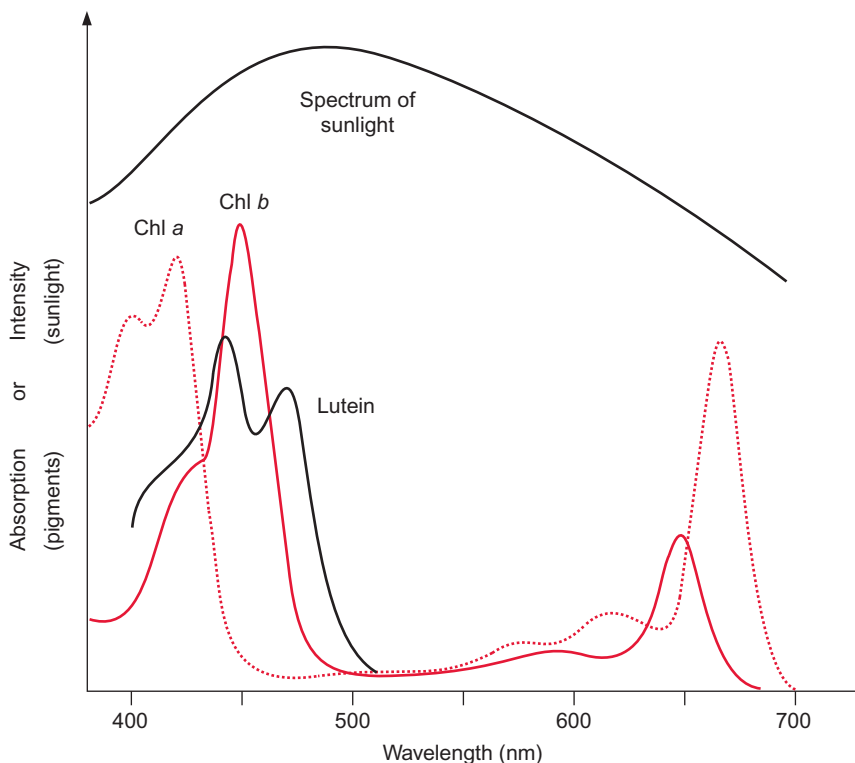
In photosynthesis of a green plant, light is collected primarily by **chlorophylls**, pigments that absorb light at a wavelength below 480 nm and between 550 and 700 nm (Fig. 2.3). When white sunlight falls on a chlorophyll layer, the green light with a wavelength between 480 and 550 nm is not absorbed, but is reflected. This is why plant chlorophylls and whole leaves appear green.

Experiments carried out between 1905 and 1913 in Zurich and Berlin by Richard Willstätter and his collaborators led to the discovery of the

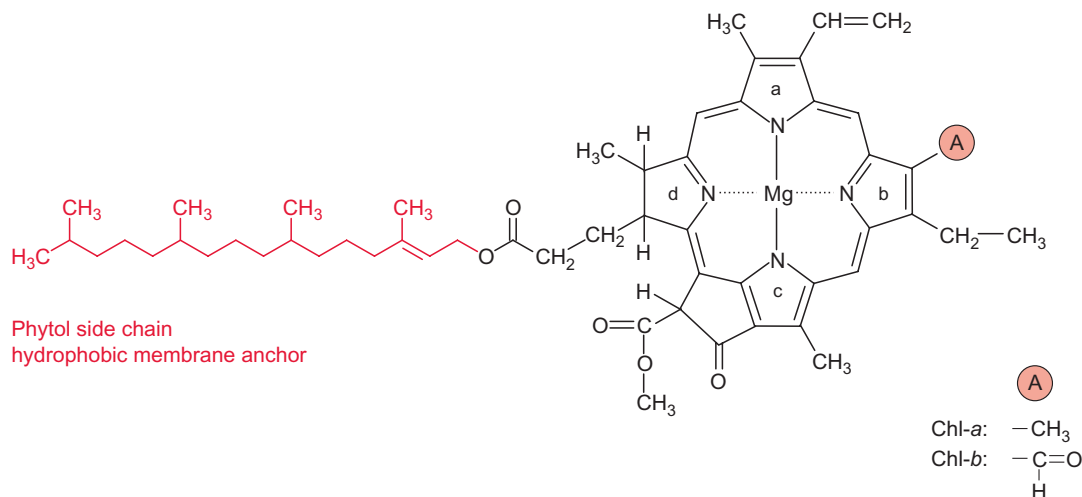
Table 2.1: The energy content and the electrochemical potential difference of photons of different wavelengths

Wavelengths (nm)	Light color	Energy content kJ/mol photons	ΔE e volt
700	Red	170	1.76
650	Bright red	183	1.90
600	Yellow	199	2.06
500	Blue green	238	2.47
440	Blue	271	2.80
400	Violet	298	3.09

Figure 2.3 Absorption spectrum of chlorophyll-*a* (chl-*a*), chlorophyll-*b* (chl-*b*) and of the xanthophyll lutein dissolved in acetone. The intensity of the sun's radiation at different wavelengths is given as a comparison.



structural formula of the green leaf pigment chlorophyll, a milestone in the history of chemistry. This discovery made such an impact that Richard Willstätter was awarded the Nobel Prize in Chemistry as early as 1915. There are different classes of chlorophylls. [Figure 2.4](#) shows the structural



formulas of chlorophyll-*a* and chlorophyll-*b* (**chl-*a***, **chl-*b***). The basic structure is a ring made of four pyrroles, a **tetrapyrrole**, which is also named **porphyrin**. Mg^{++} is present in the center of the ring as the central atom. Mg^{++} is covalently bound with two N atoms and coordinately bound to the other two atoms of the tetrapyrrole ring. A cyclopentanone is attached to ring c. At ring d a propionic acid group forms an ester with the alcohol **phytol**. Phytol consists of a long branched hydrocarbon chain with one C-C double bond. It is derived from an isoprenoid, formed from four isoprene units (section 17.7). This long hydrophobic hydrocarbon tail renders the chlorophyll highly soluble in lipids and therefore promotes its presence in the membrane phase. Chlorophyll always occurs bound to proteins. Chl-*b* contains a formyl residue in ring b instead of the methyl residue as in chl-*a*. This small difference has a large influence on light absorption. Figure 2.3 shows that the absorption spectra of chl-*a* and chl-*b* differ markedly.

In plants, the ratio chl-*a* to chl-*b* is about three to one. Only chl-*a* is a constituent of the photosynthetic reaction centers (sections 3.6 and 3.8) and therefore it can be regarded as the central photosynthesis pigment. In a wide range of the visible spectrum, however, chl-*a* does not absorb light (Fig. 2.3). This non-absorbing region is named the “**green window**.” The absorption gap is narrowed by the light absorption of chl-*b*, with its first maximum at a higher wavelength than chl-*a* and the second maximum at a lower wavelength. As shown in section 2.4, the light energy absorbed by chl-*b* can be transferred very efficiently to chl-*a*. In this way, chl-*b* enhances the plant’s efficiency for utilizing sunlight energy.

The structure of chlorophylls has remained remarkably constant during the course of evolution. Purple bacteria, probably formed more than 3 billion

Figure 2.4 Structural formula of chlorophyll-*a*. In chlorophyll-*b* the methyl group in ring b is replaced by a formyl group (A). The phytol side chain in red gives chlorophyll a lipid character.

years ago, contain as photosynthetic pigment a bacteriochlorophyll-*a*, which differs from the chl-*a* shown in Fig. 2.4 only by the alteration of one side chain and by the lack of one double bond. This, however, influences light absorption; both absorption maxima are shifted outwards and the non-absorbing spectral region in the middle is broadened. This shift allows purple bacteria to utilize light in the infrared region.

The tetrapyrrole ring not only is a constituent of chlorophyll but also has attained a variety of other functions during evolution. It is involved in methane formation by bacteria with Ni^{++} as the central atom. With Co^{++} it forms **cobalamin** (vitamin B_{12}), which participates as a cofactor in reactions in which hydrogen and organic groups change their position. With Fe^{++} instead of Mg^{++} as the central atom, the tetrapyrrole ring forms the basic structure of **hemes** (Fig. 3.24), which as cytochromes function as redox carriers in electron transport processes (sections 3.7 and 5.5) and as myoglobin or hemoglobin stores or transports oxygen in aerobic organisms. The tetrapyrrole ring in animal hemoglobin differs only slightly from the tetrapyrrole ring of chl-*a* (Fig. 2.4).

It seems remarkable that a substance that attained a certain function during evolution is being utilized after only minor changes for completely different functions. The reason for this functional variability is that the reactivity of compounds such as chlorophyll or heme is governed to a great extent by the proteins to which they are bound.

Chlorophyll molecules are bound to chlorophyll-binding proteins. In a complex with proteins the absorption spectrum of the bound chlorophyll differs considerably from the absorption spectrum of the free chlorophyll. The same applies for other light-absorbing compounds, such as carotenoids, xanthophylls, and phycobilins, which also occur bound to proteins. These complexes will be discussed in the following sections. For better discrimination in this text book, free absorbing compounds are called **chromophore** (Greek, carrier of color) and the chromophore-protein complexes are called **pigments**. Pigments are further characterized by the wavelength of their absorption maximum. Chlorophyll-*a*₇₀₀ describes a pigment of protein-chl-*a* complex with an absorption maximum of 700 nm. Another common designation is P_{700} ; this nomination leaves the nature of the chromophore open.

2.3 Light absorption excites the chlorophyll molecule

What happens when a chromophore absorbs a photon? When a photon with a certain wavelength hits a chromophore molecule that absorbs light

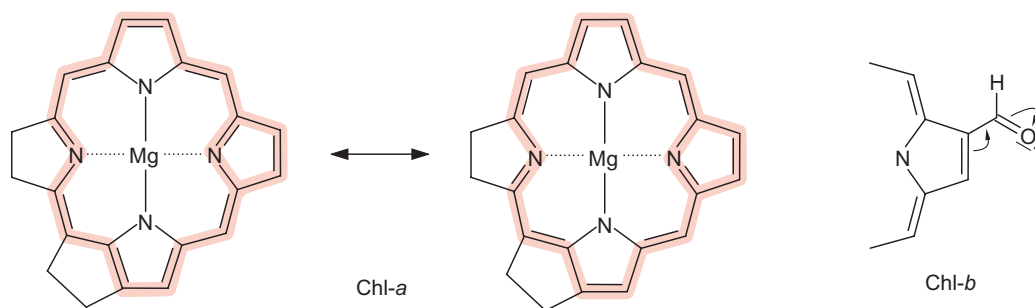


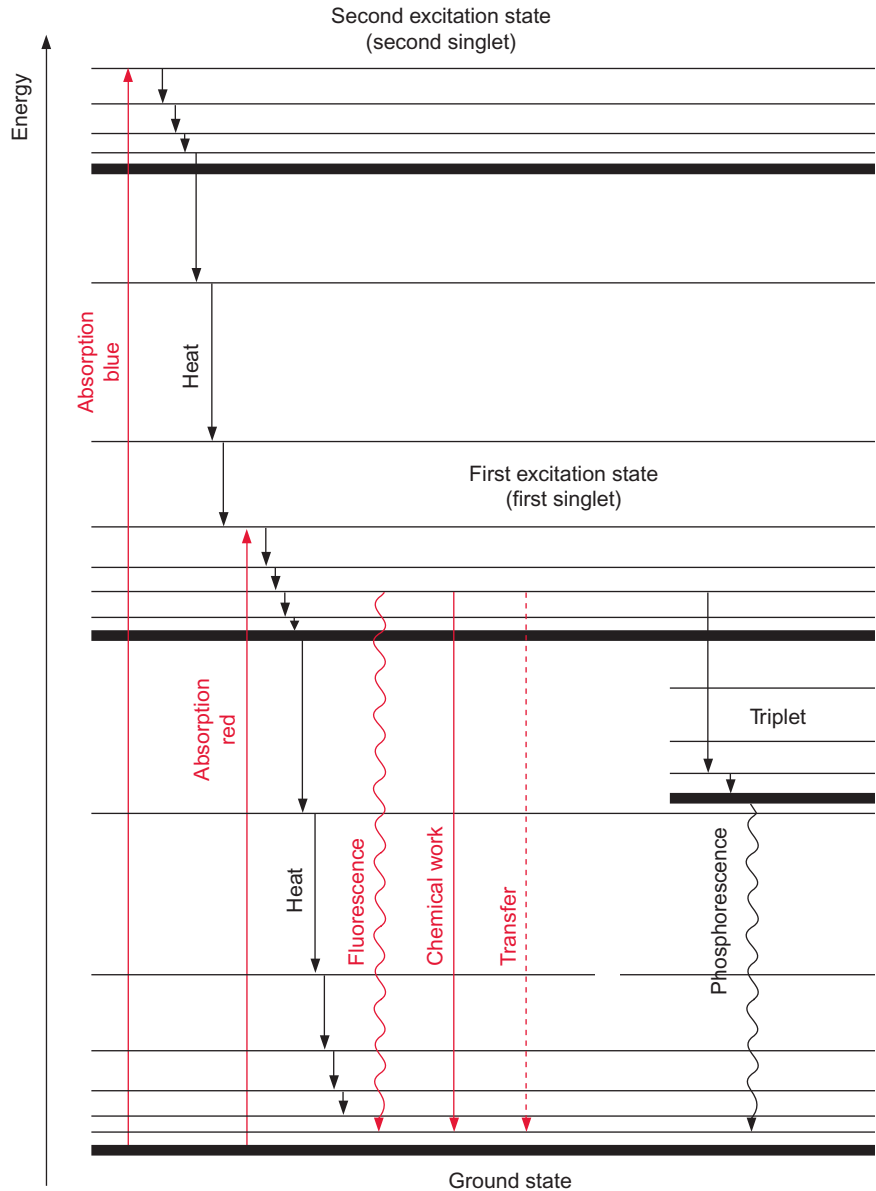
Figure 2.5 Resonance structures of chlorophyll-*a*. In the region marked red, the double bonds are not localized; the π electrons are distributed over the entire conjugated system. The formyl residue of chlorophyll-*b* attracts electrons and thus affects the π electrons of the conjugated system.

of this wavelength, the energy of the photon excites electrons and transfers them to a higher energy level. This occurs as an “all or nothing” process. According to the principle of energy conservation expressed by the first law of thermodynamics, the energy of the chromophore is increased by the energy of the photon, which results in an **excited state** of the chromophore molecule. The energy is absorbed only in discrete quanta, resulting in discrete excitation states. The energy required to excite a chromophore molecule depends on the chromophore structure. A general property of chromophores is that they contain many **conjugated double bonds**, 10 in the case of the tetrapyrrole ring of chl-*a*. These double bonds are delocalized. [Figure 2.5](#) shows two possible resonance forms.

After absorption of energy, an electron of the conjugated system is elevated to a higher orbit. This excitation state is termed **singlet**. [Figure 2.6](#) shows a scheme of the excitation process. As a rule, the higher the number of double bonds in the conjugated system, the lower the amount of energy required to produce a first singlet state. For the excitation of chlorophyll, dark red light is sufficient, whereas butadiene, with only two conjugated double bonds, requires energy-rich ultraviolet light for excitation. The light absorption of the conjugated system of the tetrapyrrole ring is influenced by the side chains. Thus, the differences in the absorption maxima of chl-*a* and chl-*b* mentioned previously can be explained by an electron attracting effect of the carbonyl side chain in ring b of chl-*b* ([Fig. 2.5](#)).

The spectra of chl-*a* and chl-*b* ([Fig. 2.3](#)) each have two main absorption maxima, showing that each chlorophyll has two main excitation states. In addition, chlorophylls have minor absorption maxima, which for the sake of simplicity will not be discussed here. The two main excitation states of chlorophyll are known as the first and second singlet ([Fig. 2.6](#)). The absorption

Figure 2.6 Schematic presentation of the excitation states of chlorophyll-*a* and their return to the ground state. The released excitation energy is converted to photochemical work, fluorescent or phosphorescent light, or dissipated into heat. This simplified scheme shows only the excitation states of the two main absorbing maxima of the chlorophylls.



maxima in the spectra are relatively broad. At a higher resolution the spectra can be shown to consist of many separate absorption lines. This fine structure of the absorption spectra is due to chlorophyll molecules that are in the ground and in the singlet states as well in **rotation** and **vibration**. In the energy scheme the various rotation and vibration energy levels are drawn as fine lines and the corresponding ground states as solid lines (Fig. 2.6).

The energy levels of the various rotation and vibration states of the ground state overlap with the lowest energy levels of the **first singlet**. Analogously, the energy levels of the first and the second singlet also overlap. If a chlorophyll molecule absorbs light in the region of its absorption maximum (blue light), one of its electrons is elevated to the **second singlet** state. This second singlet state with a half-life of only 10^{12} s is too unstable to use its energy for chemical work. The excited molecules lose energy as heat by rotations and vibrations until the first singlet state is reached. This first singlet state can also be attained by absorption of a photon of red light, which contains less energy. The first singlet state is much more stable than the second one; its half-life time is $4 \cdot 10^9$ s.

The return of the chlorophyll molecule from the first singlet state to the ground state can proceed in different ways:

1. The most important path for the conversion of the energy released upon the return of the first singlet state to the ground state is its utilization for **chemical work**. The chlorophyll molecule transfers the excited electron from the first singlet state to an electron acceptor and a positively charged chlorophyll radical chl^{\dagger} remains. This is possible since the excited electron is bound less strongly to the chromophore molecule than in the ground state. Section 3.5 describes in detail how the electron can be transferred back from the acceptor to the chl^{\dagger} radical via an electron transport chain. When the chlorophyll molecule returns to the ground state, the free energy derived from this process is conserved for chemical work. As an alternative, the electron deficit in the chl^{\dagger} radical may be replenished by another electron donor (e.g., water (section 3.6)).
2. The excited chlorophyll can return to the ground state by releasing excitation energy as light; this emitted light is named **fluorescence**. Due to vibrations and rotations, part of the excitation energy is usually lost as heat, with the result that the fluorescence light has less energy (corresponding to a longer wavelength) than the energy of the excitation light, which was required for attaining the first singlet state (Fig. 2.7).

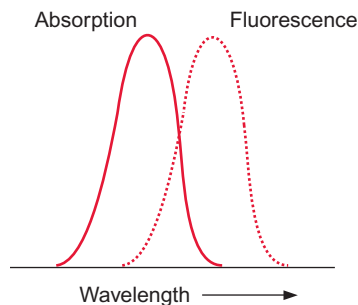


Figure 2.7 Fluorescent light has a longer wavelength than excitation light.

3. It is also possible that the return from the first singlet to the ground state proceeds in a stepwise fashion via the various levels of vibration and rotation energy, by which the energy difference is completely converted into heat.
4. By releasing part of the excitation energy as heat, the chlorophyll molecule can attain a lower energy excitation state, called the first **triplet state**. This triplet state cannot be reached directly from the ground state by excitation, since the spin of the excited electrons has been reversed. Since the probability of a reversal spin is low, the triplet state does not occur frequently. In the case of a very high excitation, however, some of the electrons of the chlorophyll molecules can reach this state. By emitting so-called **phosphorescent light**, the molecule can return from the triplet state to the ground state. Phosphorescent light is lower in energy than the light required to attain the first singlet state. The return from the triplet state to the ground state requires a reversal of the **electron spin**. As this is rather improbable, the triplet state, in comparison to the first singlet state, has a relatively long half-life time (10^{-4} to 10^{-2} s). The triplet state of the chlorophyll has no function in photosynthesis *per se*. In its triplet state, however, the chlorophyll can excite oxygen to a singlet state, whereby the oxygen becomes very reactive (reactive oxygen species, ROS, section 5.7) with a damaging effect on cell constituents. Section 3.10 describes how the plant manages to protect itself from the **harmful singlet oxygen**.
5. The return to the ground state can be coupled with the excitation of a neighboring chromophore molecule. This transfer is important for the function of the antennae and will be described in the following section.

2.4 An antenna is required to capture light

In order to excite a photosynthetic reaction center, a photon with defined energy content has to react with a chlorophyll molecule in the reaction center. The probability is very low that a photon not only has the proper energy, but also hits the pigment exactly at the site of the chlorophyll molecule. Therefore efficient photosynthesis is possible only when the energy of photons of various wavelengths is captured over a certain surface by a so-called **antenna** (Fig. 2.8). Similarly, radio and television sets could not work without an antenna.

The antennae of plants consist of a large number of protein-bound chlorophyll molecules that absorb photons and transfer their energy to the

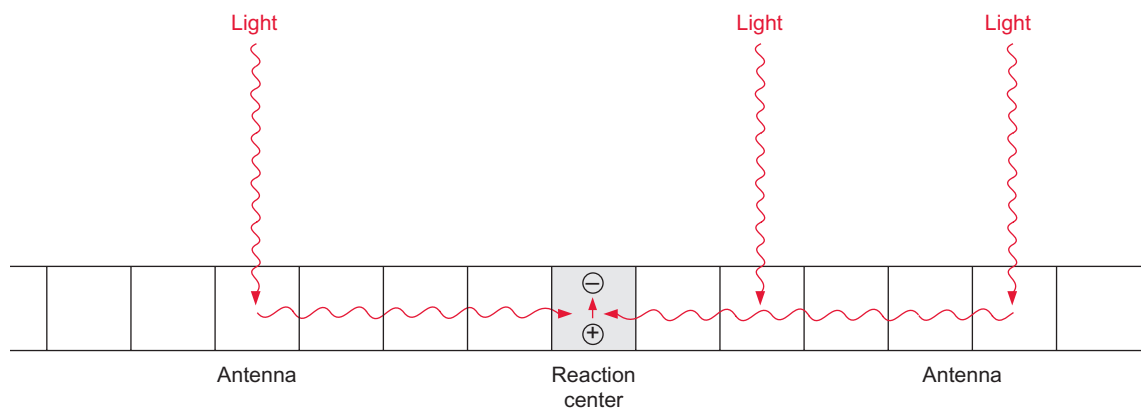


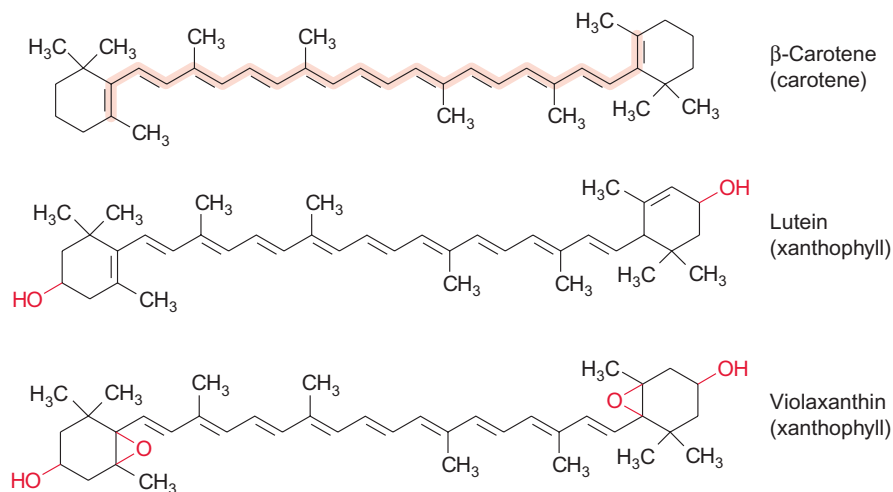
Figure 2.8 Photons are collected by an antenna and their energy is transferred to the reaction center. In this scheme the squares represent chlorophyll molecules. The excitons conducted to the reaction center cause a charge separation (section 3.4).

reaction center. Only a few thousandths of the chlorophyll molecules in the leaf are constituents of the actual reaction centers; the remainder are contained in the antennae. Observations made as early as 1932 by Robert Emerson and William Arnold in the United States indicated that the large majority of chlorophyll molecules are not part of the reaction centers. The two researchers illuminated a suspension of the green alga *Chlorella* with light pulses of $10\mu\text{s}$ duration, interrupted by dark intervals of 20 ms. Evolution of oxygen was used as a measure for photosynthesis. The light pulses were made so short that chlorophyll could undergo only one photosynthetic excitation cycle and a high light intensity was chosen in order to achieve maximum oxygen evolution. Apparently the photosynthetic apparatus was thus saturated with photons. Analysis of the chlorophyll content of the algae suspension showed that under saturating conditions only one molecule of O_2 was formed per 2,400 chlorophyll molecules.

In the following years Robert Emerson refined these experiments and was able to show when pulses were applied at very low light intensity, the amount of oxygen formed increased proportionally with the light intensity. From this it was calculated that the release of one molecule of oxygen had a minimum quantum requirement of about **eight photons**. These results settled a long scientific dispute with Otto Warburg, who had concluded from his experiments that only four photons are required for the evolution of one molecule of O_2 . Later it was recognized that each of the two reaction centers requires four photons for the formation of O_2 . Moreover, the results of Emerson and Arnold allowed the calculation that about **300 chlorophyll molecules** are associated with one reaction center. These are constituents of the **antennae**.

The antennae contain additional accessory pigments to utilize those photons where the wavelength corresponds to the “**green window**” between the absorption maxima of the chlorophylls. In higher plants these pigments

Figure 2.9 Structural formula of β -carotene and of two xanthophylls (lutein and violaxanthin). Due to the conjugated double bounds of the isoprenoid chain, these molecules absorb light and also have lipid character.



are carotenoids, mainly **xanthophylls**, including lutein and the related violaxanthin as well as **carotenes** such as β -carotene to name the major compound (Fig. 2.9). Moreover, an important function of these carotenoids in the antennae is to prevent the formation of the harmful triplet state of the chlorophylls (section 3.10). Important constituents of the antennae in cyanobacteria are **phycobilins**, which will be discussed at the end of this chapter.

How is the excitation energy of the photons captured in the antennae and transferred to the reaction centers?

The transfer of energy in the antennae via electron transport from chromophore to chromophore in a sequence of redox processes, as in the electron transport chains of photosynthesis or of mitochondrial respiration (Chapters 3 and 5), could be excluded, since such an electron transport would need considerable activation energy. This is not the case, since a flux of excitation energy can be measured in the antennae at temperatures as low as 1 K. At these low temperatures light absorption and fluorescence still occur, whereas chemical processes catalyzed by enzymes are completely inactive. Under these conditions the energy transfer in the antennae proceeds according to a mechanism that is related to those of light absorption and fluorescence.

When chromophores are positioned very close to each other, the quantum energy of an irradiated photon is transferred from one chromophore to the next. One quantum of light energy is named a **photon**, one quantum of excitation energy transferred from one molecule to the next is termed

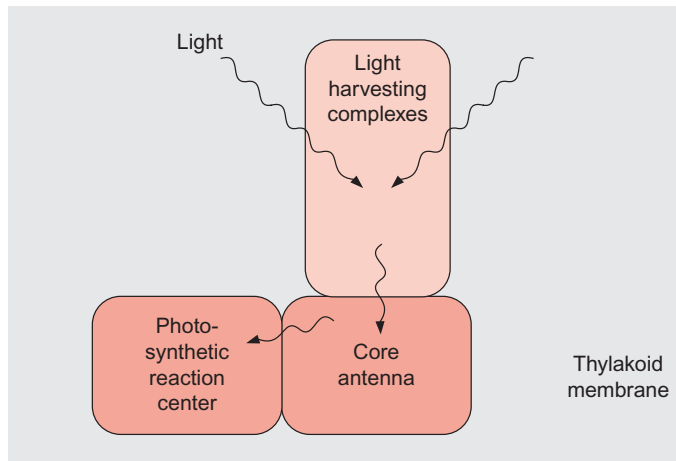


Figure 2.10 Schematic presentation of a higher plant antenna.

an **exciton**. A prerequisite for the transfer of excitons is a specific positioning of the chromophores. This is arranged by proteins, and therefore the chromophores of the antennae always occur as protein complexes.

The antennae of plants consist of an inner part and an outer part (Fig. 2.10). The outer antenna, formed by the **light harvesting complexes (LHCs)**, collects the light. The inner antenna, consisting of the **core complexes**, is an integral constituent of the reaction centers; it also collects light and conducts the excitons that were collected in the outer antenna to the photosynthetic reaction centers.

The LHCs are composed of polypeptides, which bind chl-*a*, chl-*b*, xanthophylls, and carotenes. These proteins, termed **LHC polypeptides**, are encoded in the nucleus. A plant contains many different LHC polypeptides. In a tomato, for instance, at least 19 different genes for LHC polypeptides have been found, which are very similar to each other and are members of a multigene family. They are homologous, as they have all evolved from a common ancestor.

Plants contain two reaction centers, which are arranged in sequence: a reaction center of photosystem II (**PS II**), which has an absorption maximum at 680 nm, and a photosystem I (**PS I**) with an absorption maximum at 700 nm. The function of these reaction centers will be described in sections 3.6 and 3.8. Both photosystems are composed of different LHCs.

The function of an antenna is illustrated by the antenna of photosystem II

The antenna of the PS II reaction center contains primarily four LHCs termed LHC-II*a-d*. The main component is **LHC-II*b***; it represents 67% of

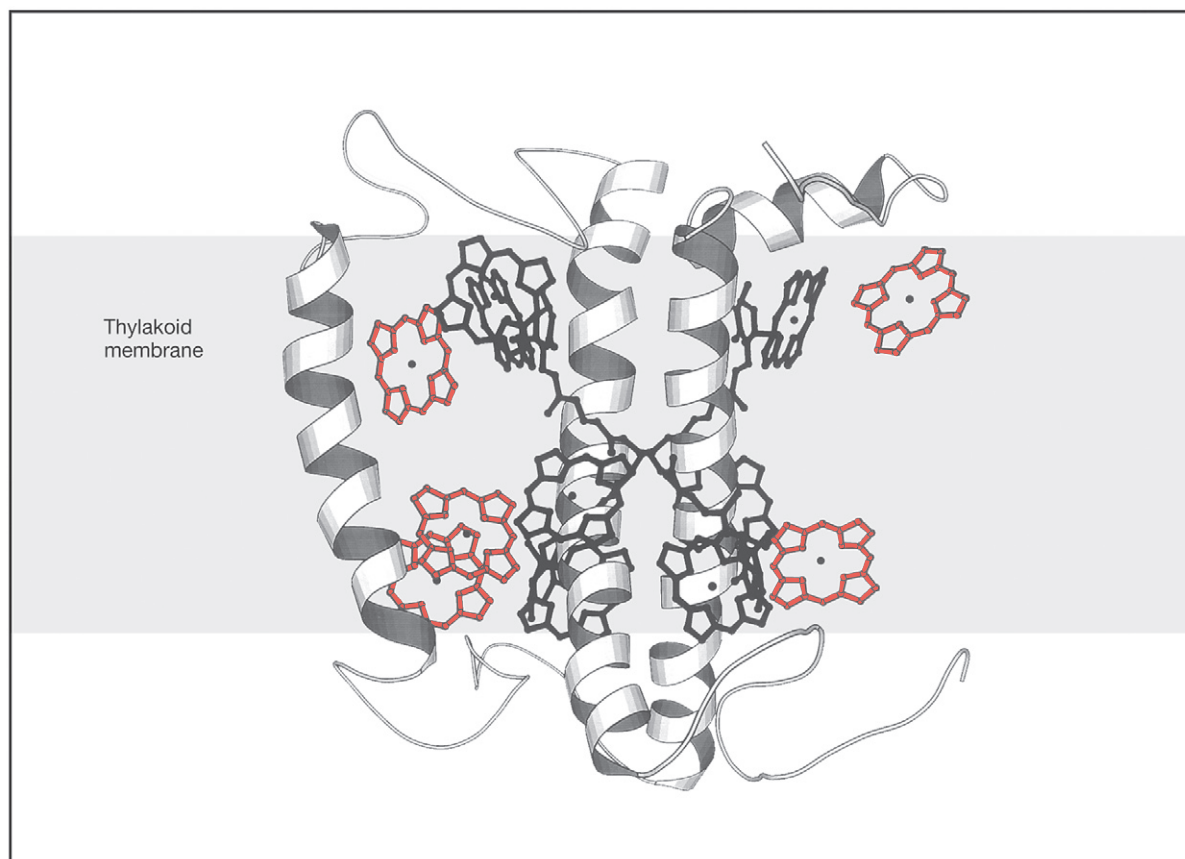
Table 2.2: Composition of the LHC-II*b*-monomer

Peptide:	232 amino acids
Lipids:	1 phosphatidylglycerol, 1 digalactosyldiacylglycerol
Chromophores:	8 chl- <i>a</i> , 6 chl- <i>b</i> , 2 lutein, 1 violaxanthin, 1 neoxanthin

the total chlorophyll of the PS II antenna and is the most abundant membrane protein of the thylakoid membrane, and has therefore been particularly thoroughly investigated. LHC-II*b* occurs in the membrane, most probably as a trimer. The monomer consists of a polypeptide to which four xanthophyll molecules are bound (Table 2.2). The polypeptide contains one threonine residue, which can be phosphorylated by ATP via a protein kinase. Phosphorylation regulates the activity of LHC-II (section 3.10).

There has been a breakthrough in establishing the three-dimensional structure of LHC-II*b* by electron cryomicroscopy at a temperature of 4K of crystalline layers of LHC-II*b*-trimers (Fig. 2.11). The LHC-II*b*-peptide forms three transmembrane helices. The two lutein molecules span the membrane crosswise. The other two molecules are not visible in the isolated LHC complex. The chl-*b*-molecules, where the absorption maximum in the red spectral region lies at a shorter wavelength than that of chl-*a*, are positioned in the outer region of the complexes. Only one of the chl-*a*-molecules is positioned in the outer region; the others are all present in the center. Figure 2.12 shows a vertical projection of the arrangement of the monomers to form a trimer. The chl-*a* positioned in the outer region mediates the transfer of energy to the neighboring trimers or to the reaction center. The trimers are arranged in the membrane as oligomers forming the antenna for the conductance of the absorbed excitons. The chl-*a*/chl-*b* ratio is much higher in LHC-II*a* and LHC-II*c* than in LHC-II*b*. Most likely LHC-II*a* and LHC-II*c* are positioned between LHC-II*b* and the reaction center.

Figure 2.13 shows a hypothetical scheme of the array of the PS II antenna. The outer complexes, consisting of LHC-II*b*, are present at the periphery of the antenna. The excitons captured by chl-*b* in LHC-II*b* are transferred to chl-*a* in the center of the LHC-II*b* monomers and are then transferred further by chl-*a* contacts between the trimers to the inner antennae complexes. The inner complexes are connected by small chlorophyll containing subunits to the core complex. This consists of the antennae proteins CP 43 and CP 47, which are closely attached to the reaction center (Fig. 3.22), and each containing about 15 chl-*a* molecules. Since the absorption maximum of chl-*b* is at a lower wavelength than that of chl-*a*, the



transfer of excitons from chl-*b* to chl-*a* is accompanied by loss of energy as heat. This promotes the flux of excitons from the periphery to the reaction center. The connection between the outer LHCs (LHC-II*b*) and the PS II can be interrupted by phosphorylation. In this way the actual size of the antenna can be adjusted to the intensity of illumination (section 3.10).

Photosystem I contains fewer LHCs than photosystem II (section 3.8) since its core antenna is larger than in PS II. The LHCs of PS I are similar to those of PS II. Sequence analysis shows that LHC-I and LHC-II stem from a common ancestor. It has been suggested that in the phosphorylated state LHC-II*b* can also function as an antenna of PS I (section 3.10).

There are two mechanisms for the movement of excitons. The excitons may be delocalized by distribution over the whole chromophore molecules. On the other hand, excitons may also initially be present in a certain chromophore molecule and subsequently transferred to a more distant chromophore. This process of exciton transfer has been termed the **Förster mechanism**. The transfer of excitons between closely neighboring chlorophyll

Figure 2.11 Sterical arrangement of the LHC-II*b* monomer in the thylakoid membrane, viewed from the side. Three α -helices of the protein span the membrane. Chlorophyll-*a* (black tetrapyrrole ring) and chlorophyll-*b* (red tetrapyrrole ring) are oriented almost perpendicularly to the membrane surface. Two lutein molecules (black carbon chain) in the center of the complex act as an internal cross brace. (By courtesy of W. Kühlbrandt, Heidelberg.)

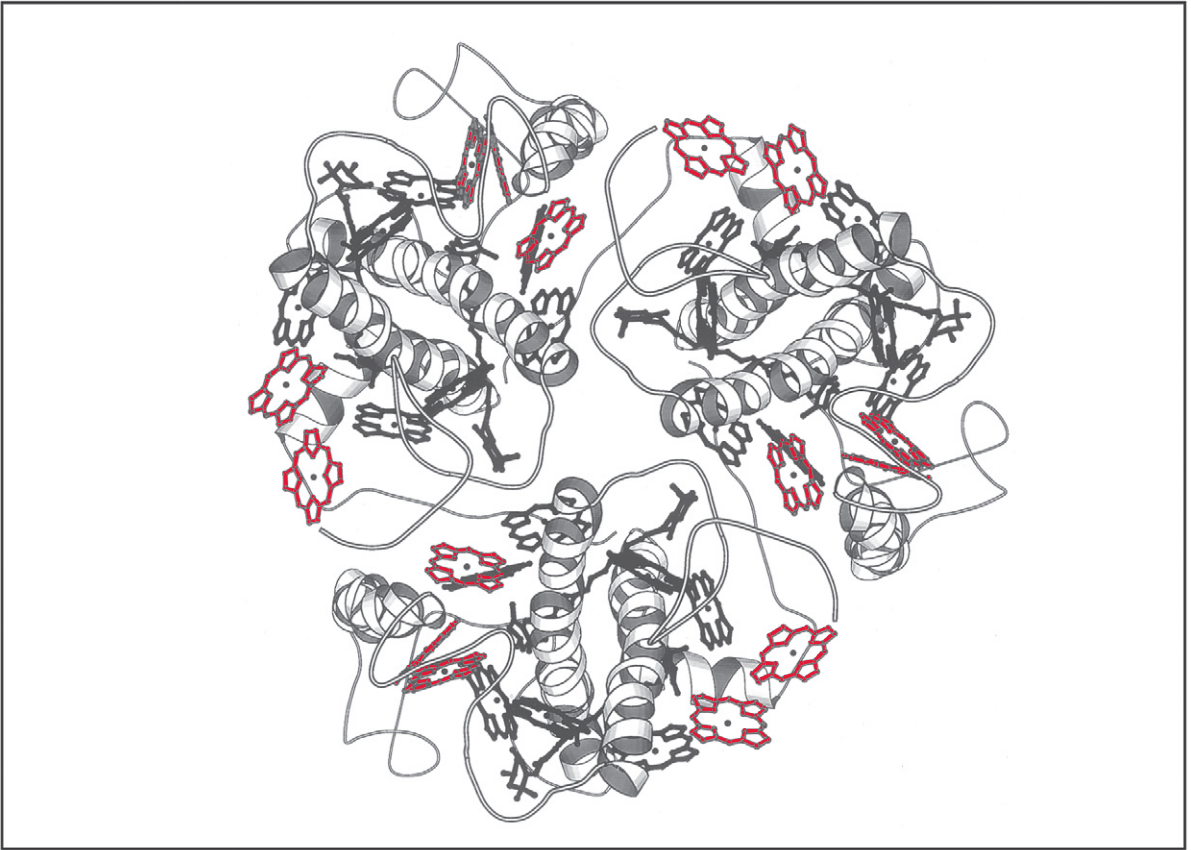


Figure 2.12 The LHC-II-trimer viewed from above from the stroma side. Within each monomer the central pair of helices forms a left-handed super coil, which is surrounded by chlorophyll molecules. The chl-*b* molecules (red) are positioned at the side of the monomers. (By courtesy of W. Kühlbrandt, Heidelberg.)

molecules within an LHC complex probably proceeds via **delocalized electrons**, and the transfer between the LHCs and the reaction center occurs via the Förster mechanism. Absorption measurements with ultrafast laser technique have shown that the exciton transfer between two chlorophyll molecules proceeds within 0.1 ps (10^{-13} s). Thus the velocity of the exciton transfer in the antennae is much faster than the charge separation in the reaction center (≈ 3.5 ps) (section 3.4). The reaction center functions as an **energy trap** for excitons present in the antenna.

Phycobilisomes enable cyanobacteria and red algae to carry out photosynthesis even in dim light

Cyanobacteria and red algae possess antennae structures that can collect light of very low intensity. These antennae are arranged as complexes on top of the membrane near the reaction centers of photosystem II (Fig. 2.14).

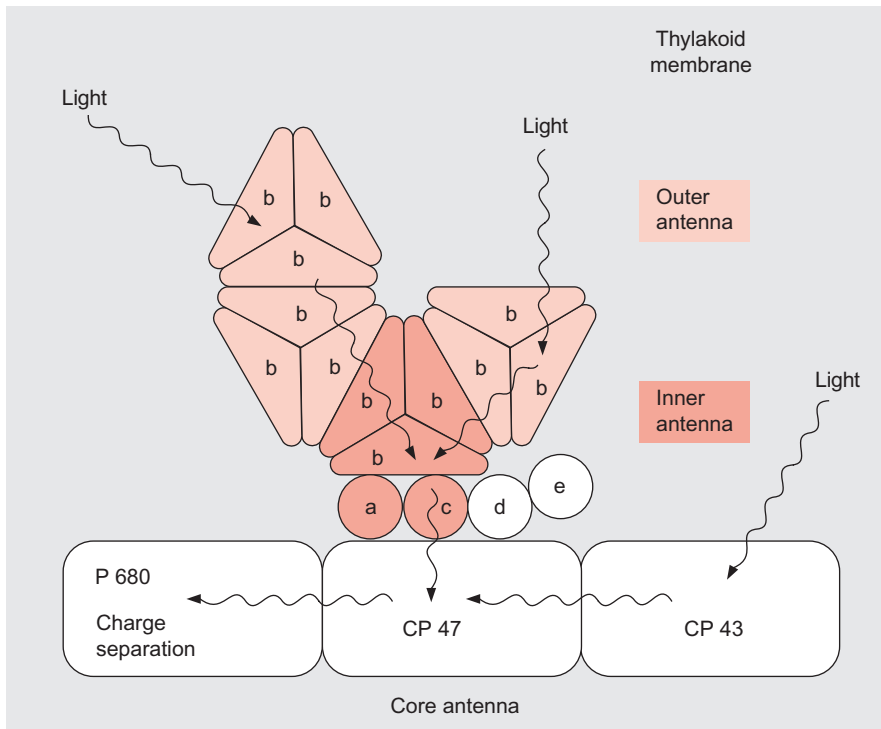


Figure 2.13 Schematic presentation of the light harvesting complexes in the antenna of photosystem II in a plant viewed from above (after Thornber); (a) LHC-IIa, (b) LHC-IIb. The inner antenna complexes are linked to the core complex by LHC-IIa and LHC-IIc (c) monomers. The function of the LHC-IIe (d) and LHC-IIe (e) monomers is not entirely known.

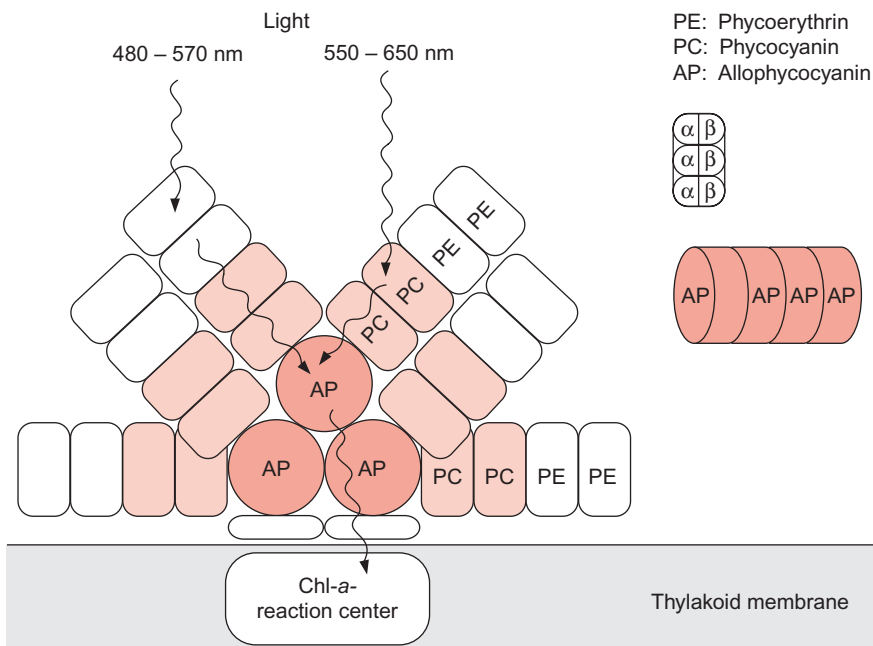


Figure 2.14 Schematic presentation of a side view of the structure of a phycobilisome. Each of the units shown consists of three α - and three β -subunits. (After Bryanth.)

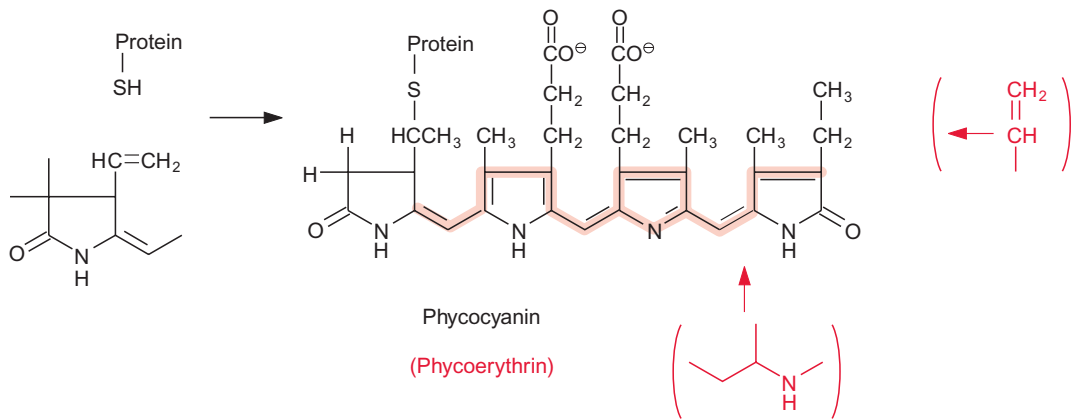


Figure 2.15 Structural formula of the biliproteins that are present in the phycobilisomes, phycocyanin (black), and phycoerythrin (structural differences from phycocyanin are shown in red). The corresponding chromophores, phycocyanobilin and phycoerythrobilin are covalently bound to proteins via thioether linkages between the SH group of a cysteine residue of the protein and the vinyl group of the chromophore. The conjugated double bonds (red) show molecules with pigment-like character.

These complexes, termed **phycobilisomes**, consist of proteins (**phycobiliproteins**), which are covalently linked with phycobilins. **Phycobilins** are open-chained tetrapyrroles and therefore are structurally related to the chlorophylls. Open-chained tetrapyrroles are also contained in bile, which explains the name *-bilin*. The phycobilins are linked to the protein by a thioether bond between an SH-group of the protein and the vinyl side chain of the phycobilin. The protein **phycoerythrin** is linked to the chromophore **phycoerythrobilin**, and the proteins **phycocyanin** and **allophycocyanin** to the chromophore **phycocyanobilin** (Fig. 2.15). The basic structure in the phycobiliproteins consists of a heterodimer composed of α - and β -subunits. Each of these protein subunits binds one to four phycobilins as a chromophore. Three of these heterodimers aggregate to a trimer $(\alpha, \beta)_3$ and thus form the actual building block of a phycobilisome. Specific linker polypeptides function as “mortar” between the building blocks.

Figure 2.14 shows the structure of a phycobilisome. The phycobilisome is attached to the membrane by anchor proteins. Three aggregates of four to five $(\alpha, \beta)_3$ units form the core. This core contains the chromophore allophycocyanin (AP) to which cylindrical rod like structures are attached, each with four to six building blocks. The inner units contain mainly phycocyanine (PC) and the outer ones phycoerythrin (PE). The function of this structural organization is illustrated by the absorption spectra of

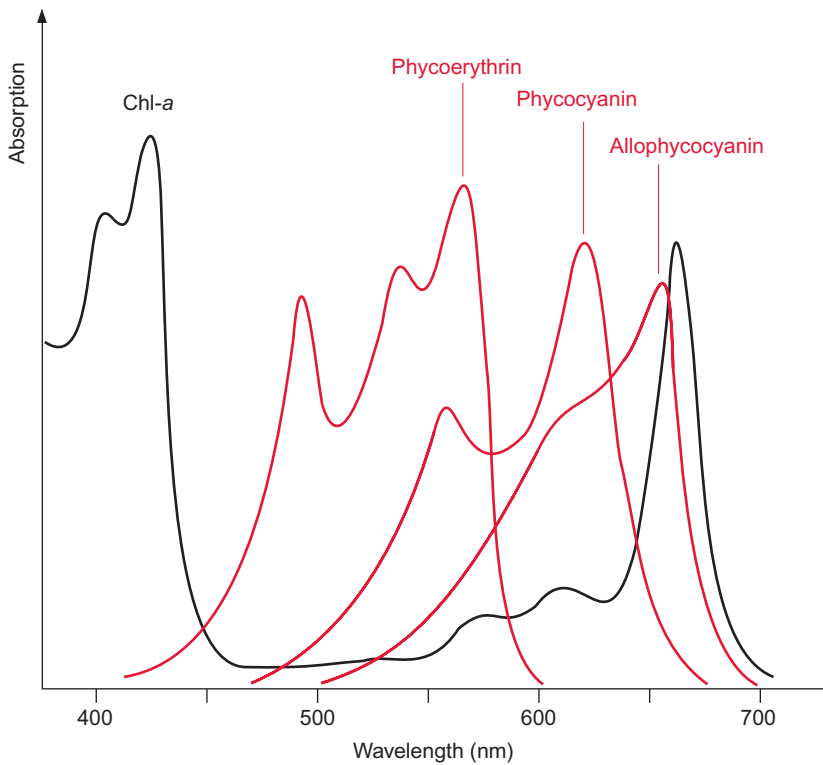


Figure 2.16
Absorption spectra of the phycobiliproteins phycoerythrin, phycocyanin, and allophycocyanin. For the sake of comparison chlorophyll-*a* is shown.

the various biliproteins shown in [Figure 2.16](#). The light of shorter wavelength is absorbed in the periphery of the rods by phycoerythrin and the light of longer wavelength in the inner regions of the rods by phycocyanin. The core transfers the excitons to the reaction center. The principle of spatial distribution between the short wavelength absorbing pigments at the periphery and the long wavelength absorbing pigments in the center is also implemented for the PS II antennae of higher plants ([Fig. 2.10](#)).

Due to the phycobiliproteins, phycobilisomes are able to absorb green light very efficiently ([Fig. 2.16](#)), thus allowing cyanobacteria and red algae to survive in deep waters with low light intensities. At these depths, due to the “green window” of photosynthesis ([Fig. 2.3](#)), only green light is available, as the light of the other wavelengths is absorbed by green algae living in the upper regions of the water column. The algae in the deeper regions are obliged to invest a large portion of their cellular matter in phycobilisomes in order to carry out photosynthesis at this very low light intensity and at distinct wavelengths. Biliproteins can amount to 40% of the total cellular protein of the algae. These organisms undertake an extraordinary expenditure to collect enough light for survival.

Further reading

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