

# Algal Pigments

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A wide variety of pigments in algae allow the absorption of light energy which is then converted to chemical energy in the photosynthetic membranes. Such variety allows them to adapt successfully to environments of differing light conditions.

## Introduction

Algae contain a wide variety of pigments that absorb light for photosynthesis. Aquatic light environments can be extremely variable. The variety of pigments in algae has contributed to their successful acclimation to light environments of different quality and intensity. The major photosynthetic pigments are chlorophylls, carotenoids and phycobiliproteins. Chlorophyll *a* is common to all oxygenic photosynthetic organisms including land plants which, like green algae, also contain chlorophyll *b*. In chromophytes, chlorophyll *c* and the carotenoid fucoxanthin are also important light-harvesting pigments. Phycobiliproteins are major antenna pigments in cyanobacteria, red algae and cryptophytes. In dinoflagellates the carotenoid peridinin together with chlorophyll *a* and *c* are the major light-gathering pigments.

## Pigment Complexes and Cellular Localization

The light-harvesting pigments in algae and plants are contained within multiprotein complexes which orient and coordinate pigment interaction and funnel the absorbed light energy to distinct photosynthetic reaction centres (RC) located within the photosynthetic membranes (thylakoids). The chromophores of all pigments are invariably bound to proteins that constitute the photosystem I and photosystem II complexes. Chlorophyll *a* (Chl *a*) is the predominant pigment in reaction centres with approximately 75–100 Chl *a* and 12–15  $\beta$ -carotene molecules contained in the core photosystem I reaction centre (RC1). The photosystem II reaction centre complex (RC2) has around 45 Chl *a* and 10  $\beta$ -carotene molecules within the core and the core-antennae. Major classes of antennae complexes are the LHCs (light-harvesting chlorophyll/carotenoid-binding complexes) directly attached to the reaction centres in the thylakoids, and the phycobilisomes, which are peripheral supramolecular complexes of phycobiliproteins that project into the stromal region and are functionally attached to RC2 (Figure 1). Identification of pigments participating in

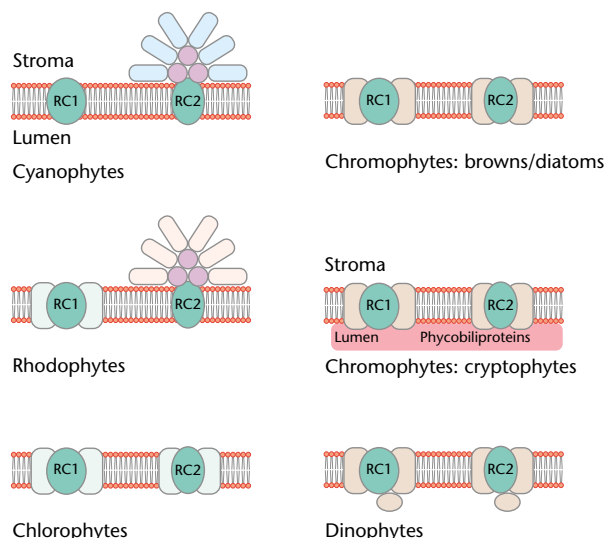
## Secondary article

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photosynthesis has required making 'action spectra' of whole cells and comparing them with the absorption spectra of isolated pigments. Because of the considerable spectral overlap of many pigments, it may be difficult to confirm the involvement of a specific pigment.

Green plants, largely based on land, have a limited repertoire of pigment types compared to algae living in aquatic environments where penetrating wavelengths of light may be variously attenuated as they pass through the water column. The LHCs in these aquatic organisms show a great diversity of pigment types including Chl *a*, *b*, *c*<sub>1</sub>, *c*<sub>2</sub>, *c*<sub>3</sub>, and *d* and a multitude of carotenoids (Table 1). Most



**Figure 1** A variety of chlorophyll/carotenoid-binding LHCs and phycobilisomes are associated with reaction centres of photosystem I (RC1) and II (RC2): in cyanophytes (e.g. *Synechococcus*) phycobilisomes transfer energy initially to RC2, and LHCs are lacking; rhodophytes (e.g. *Porphyridium*) have Chl *a*/car LHCs associated only with RC1, and phycobilisomes only with RC2. In chlorophytes (e.g. *Pisum*) distinct Chl *a*/*b*/car LHCs are associated with each RC. In chromophytes (e.g. *Cylindrotheca*) distinct Chl *a/c*-car LHCs occur with RC1 and RC2. In cryptophytes (e.g. *Cryptomonas*) phycobiliproteins in the lumen transfer energy to Chl *a*, but not to Chl *c*. Both Chl types are associated with RC1 and RC2. Dinophytes (e.g. *Gonyaulax*) have a luminal peridinin–Chl *a/c* complex and an integral Chl *a/c* complex in thylakoids.

**Table 1** Pigments of photosynthetic oxygenic organisms

| Sample organisms                  | Chlorophylls                                     | Carotenoids                    | Phycobiliproteins |
|-----------------------------------|--|--------------------------------|-------------------|
| <b>Cyanophytes:</b>               |  |                                |                   |
| <i>Acaryochloris</i>              | <i>a, d</i> , MgDvp                              | zea, $\alpha$ -car             | apc, pc           |
| <i>Fremyella</i>                  | <i>a</i>   | zea, $\beta$ -car <sup>a</sup> | apc, pc, pe       |
| <i>Synechococcus</i>              | <i>a</i>   | zea                            | apc, pc           |
| <b>Prochlorophytes:</b>           |  |                                |                   |
| <i>Prochloron</i>                 | <i>a, b</i> , MgDvp                              | zea                            | –                 |
| <i>Prochlorococcus</i>            | Dva, Dvb   | zea, $\alpha$ -car             | pe <sup>b</sup>   |
| <i>Prochlorothrix</i>             | Dva, Dvb   | zea                            | –                 |
| <b>Chlorophytes:</b>              |  |                                |                   |
| <i>Chlamydomonas</i>              | <i>a, b</i>                                      | lor, lut, vio, zea             | –                 |
| <i>Codium</i>                     | <i>a, b</i>                                      | lut, siph, sixa                | –                 |
| <i>Euglena</i>                    | <i>a, b</i>                                      | dia, dit, neo                  | –                 |
| <i>Mantoniella</i>                | <i>a, b</i> , MgDvp                              | ant, lut, neo, pras, zea       | –                 |
| <i>Pisum</i> (pea)                | <i>a, b</i>                                      | lut, neo, vio, zea             | –                 |
| <b>Rhodophytes:</b>               |  |                                |                   |
| <i>Gigartina</i>                  | <i>a, d</i>                                      | lut, neo, zea                  | apc, pc, pe       |
| <i>Porphyridium</i>               | <i>a</i>   | zea                            | apc, pc, pe       |
| <b>Chromophytes:</b>              |  |                                |                   |
| Phaeo: <i>Fucus, Laminaria</i>    | <i>a, c</i> <sub>1</sub> , <i>c</i> <sub>2</sub> | fuco                           | –                 |
| Bacillaria: <i>Odontella</i>      | <i>a, c</i> <sub>1</sub> , <i>c</i> <sub>2</sub> | fuco                           | –                 |
| <i>Phaeodactylum</i>              | <i>a, c</i> <sub>1</sub> , <i>c</i> <sub>2</sub> | dia, fuco                      | –                 |
| Chryso: <i>Giraudyopsis</i>       | <i>a, c</i> <sub>1</sub> , <i>c</i> <sub>2</sub> | fuco, zea                      | –                 |
| <i>Ochromonas</i>                 | <i>a, c</i>                                      | ant                            | –                 |
| Raphido: <i>Heterosigma</i>       | <i>a, c</i> <sub>1</sub> , <i>c</i> <sub>2</sub> | dia, dino, fuco                | –                 |
| Xantho: <i>Pleurochloris</i>      | <i>a, c</i>                                      | dia, het, vau                  | –                 |
| Hapto: <i>Pavlova, Isochrysis</i> | <i>a, c</i> <sub>1</sub> , <i>c</i> <sub>2</sub> | fuco                           | –                 |
| Eustigma: <i>Nannochloropsis</i>  | <i>a</i>   | vio, vau                       | –                 |
| Crypto: <i>Chroomonas</i>         | <i>a, c</i> <sub>2</sub>                         | allo                           | pc                |
| <i>Cryptomonas</i>                | <i>a, c</i> <sub>2</sub>                         | allo                           | pe                |
| <b>Dinophytes:</b>                |  |                                |                   |
| <i>Amphidinium</i>                | <i>a, c</i> <sub>2</sub>                         | dia, dino, dit, per            | –                 |
| <i>Symbiodinium</i>               | <i>a, c</i> <sub>2</sub>                         | dia, per                       | –                 |

$\alpha$ -car,  $\alpha$ -carotene; allo, alloxanthin; apc, allophycocyanin; ant, antheraxanthin;  $\beta$ -car,  $\beta$ -carotene; dia, diadinoxanthin; dit, diatoxanthin; dino, dinoxanthin; Dva, divinyl chlorophyll *a*; Dvb, divinyl chlorophyll *b*; fuco, fucoxanthin; het, heteroxanthin; lor, loroxanthin; lut, lutein; MgDvp, magnesium divinyl pheophorbirin monomethyl ester; neo, neoxanthin; pc, phycocyanin; pe, phycoerythrin; per, peridinin; pras, prasinoxanthin; siph, siphonoin; sixa, siphonaxanthin; vau, vaucherixanthin; vio, violaxanthin; zea, zeaxanthin.

<sup>a</sup> $\beta$ -Carotene occurs in reaction centres of most cyanobacteria, algae and plants.

<sup>b</sup>In *P. marinus* CCMP 1375.

LHCs exist as integral multipetide complexes within thylakoid membranes that can only be released upon treatment with detergents. Models representing the localization of the antennae complexes and RCs are shown in

**Figure 1.** In Chl *a/c*-containing algae the association of LHCs with the photosystems is not as well established as for the other groups.

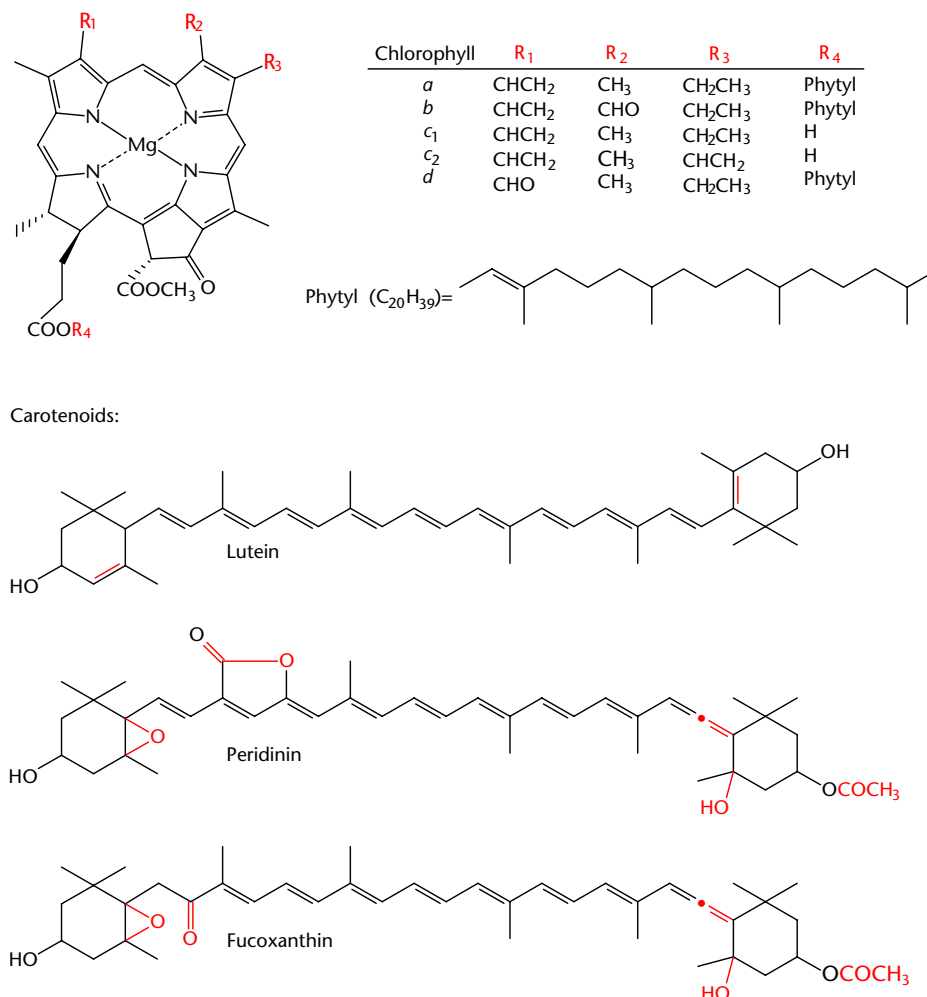
## Chlorophylls

Chlorophylls are green pigments with a porphyrin-like ring structure, a central magnesium atom (**Figure 2**), and usually a long hydrophobic tail. The molecular structure of these compounds varies by one of several side-chain substitutions that affect the absorption characteristics. Chlorophyll *a* occurs in all oxygenic organisms (**Table 1**). In native pigment–protein complexes it has an absorption maximum at about 438 nm in the blue wavelength region and another in the red region at 670 nm, and it has a fluorescence emission at 677 nm (excited at 440 nm). For chlorophyll extracts in many organic solvents the spectral maxima shift toward shorter wavelengths (as in **Figure 3a**): Chl *a*, 430 and 662 nm; Chl *b*, 457 and 645 nm; Chl *c*<sub>1</sub>, 446 and 628 nm; Chl *d*, 445 and 693 nm. The extinction at the red absorption maximum for the various Chl *c* forms is much lower than those of other chlorophylls. *In vivo* the

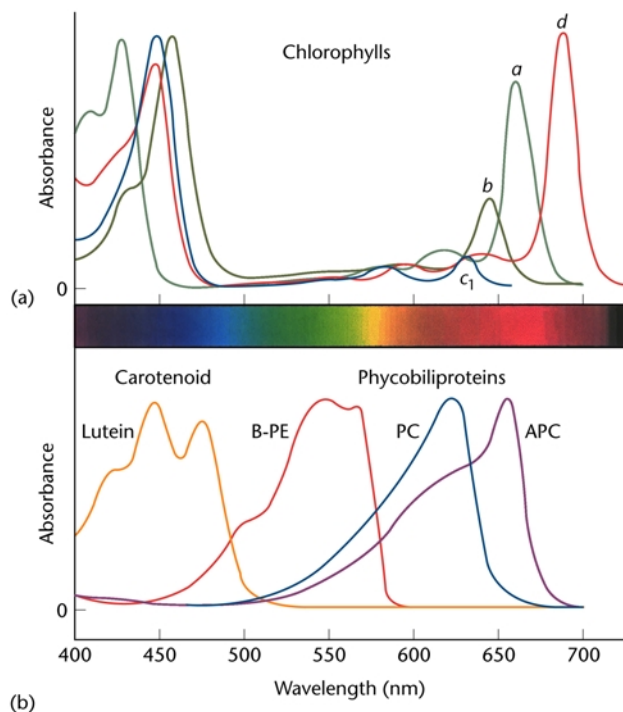
transfer of energy is always unidirectional to Chl *a* from either Chl *b* or *c*. The existence of Chl *d*, first found in a red alga, was in doubt until recently when it was found to be a significant pigment in many red algae and in a prokaryotic alga (*Acaryochloris*). In addition to Chl *a* and *b* some prokaryotes, especially members of the prochlorophytes (**Table 1**), may also contain divinyl Chl *a* and *b*, or the Chl *c*-like MgDvp (magnesium divinyl pheoporphylin monomethyl ester). Such Chl forms are probably biosynthetic intermediates and may represent vestiges of evolutionary development.

## Carotenoids

Carotenoid pigments are ubiquitous in photosynthetic organisms. The major functions of these yellow and orange



**Figure 2** Molecular structures of chlorophylls and photosynthetically important carotenoids. Portions highlighted in red indicate the specific side-chains of the individual chlorophylls, and the differences between lutein, peridinin and fucoxanthin.



**Figure 3** Photosynthetic pigments absorb in the visible range of the electromagnetic spectrum. (a) Spectra of chlorophylls *a* and *b* (in acetone), *c*<sub>1</sub> (in diethyl ether), and *d* (in acetone). (b) Spectra of phycobiliproteins B-PE (phycocerythrin), PC (phycocyanin) and APC (allophycocyanin) (in 0.1 mol L<sup>-1</sup> phosphate buffer). These water-soluble pigments are present in many rhodophytes and cyanobacteria. Lutein (in acetone) is a common carotenoid in green plants.

pigments in photosynthesis are to act as accessory pigments and to serve in protection against photooxidation from excess light absorbed by the light-harvesting antenna pigments. They also serve to stabilize the structural integrity of LHCs. Their importance in photosynthetic tissues is critical because chlorophyll cannot accumulate when the synthesis of cyclic carotenoids, such as  $\beta$ -carotene, is inhibited. Biosynthesis of carotenoids begins with a five-carbon compound (isopentenyl pyrophosphate) and various enzymatic steps lead to formation of a forty-carbon backbone unit with alternating saturated and unsaturated bonds. Photosynthetically important carotenoids are bicyclic (**Figure 2**) and are either derived from  $\alpha$ -carotene (one  $\beta$  and one  $\epsilon$  ring) or  $\beta$ -carotene (two  $\beta$  rings).  $\beta$ -Carotene is the most prevalent carotenoid in the RCs of cyanobacteria, eukaryotic algae and plants. Xanthophylls (oxygenated carotenoids) are localized in LHCs, where they function in photoprotection, and/or as accessory pigments. Lutein (**Figure 2**) is a major accessory pigment in higher plants, several green algae and some red algae. In the majority of dinophytes the brownish-coloured peridinin is attached to LHC proteins, where in conjunction with Chl *a* or *c* it serves as the major light-harvesting carotenoid (**Figure 2**). Fucoxanthin is the predominant

carotenoid in diatoms and brown algae (**Table 1**) and occurs in LHC complexes together with Chl *a* or *c*. Whereas many types of xanthophylls exist, of which the more common are given in **Table 1**, the role of many xanthophylls is not known. Also, it is evident from mutant studies that accumulation of a specific xanthophyll is not necessarily essential: substitutions of one type for another are possible, without any obvious deleterious effects on the organism.

In organic solvents, algal carotenoids typically exhibit a triple peak absorption spectrum in the 400–500 nm region (**Figure 3b**) where chlorophyll also absorbs. This spectral overlap and the generic carotenoid absorption spectrum together make it difficult to identify specific carotenoid pigments and evaluate their contribution to photosynthesis. *In vivo*, absorption spectra of the carotenoids are substantially shifted toward the red by 20–30 nm (or more) when bound to the LHC polypeptides.

## Phycobiliproteins and Phycobilisomes

Phycobiliproteins are water-soluble photosynthetic accessory pigments that upon cell breakage are readily released and produce vibrant blue- or red-coloured solutions. When attached to the thylakoids, usually as constituents of phycobilisomes, they transfer energy to chlorophyll *a*. Phycobiliproteins occur in cyanobacteria, rhodophytes, cryptophytes and sometimes in prochlorophytes (**Table 1**). The principal chromophore types are phycocyanobilin, mainly present in phycocyanins and allophycocyanins, and phycoerythrobilin and phycourobilin in phycoerythrins. Chemically the chromophores are linear tetrapyrroles that are covalently bound to the apoprotein by thioether linkages to one or two rings (rings A/D). Amino acid sequences of the apoproteins show that the chromophores are always attached to cysteine residues and that the sequences surrounding the chromophores tend to be very highly conserved.

The aqua-coloured allophycocyanins, absorbing at the longest wavelength (650 nm, and a 660 nm fluorescence emission) (**Figure 3b**), are located in the centre of phycobilisomes in rhodophytes and cyanobacteria (**Figure 1**), and provide the best spectral overlap with chlorophyll *a*. Deep blue-coloured phycocyanins absorb maximally around 620–630 nm and fluoresce at around 640–650 nm. Phycoerythrocyanin and R-phycocyanin are purplish in colour and are spectral variants of phycocyanin that nevertheless have the typical fluorescence emission of phycocyanins. Phycoerythrins have a pink-orange colour. They absorb maximally in the 545–560 nm range and fluoresce around 575–580 nm. Many also have a prominent 500 nm absorbance shoulder (from phycourobilin). Allophycocyanins and phycocyanins are invariably present in all phycobilisomes, but phycoerythrin is not present in all species. In phycobilisomes phycoerythrin, when

present as in *Porphyridium cruentum*, is on the periphery, with phycocyanin more internal and allophycocyanin at the centre base. Such an arrangement from a higher to a lower energy level corresponds to the following energy transfer:

Phycoerythrin⇒ Phycocyanin⇒ Allophycocyanin⇒  
Phycobilisome linker⇒ RC2

Cryptophytes are atypical in containing either phycocyanin or phycoerythrin, and lacking allophycocyanin and phycobilisome-like assemblies of phycobiliproteins. Also, the phycobiliproteins are concentrated in the thylakoid lumen (**Figure 1**). The cryptophytes are further unusual in that they are the only group that has chlorophyll *c* in addition to phycobiliproteins. It is likely that these unique combinations in cryptophytes resulted from secondary and/or tertiary endosymbiotic events.

## Phylogenetic Relatedness

Chloroplasts are thought to have derived from cyanobacteria through endosymbiosis. The plastids of chlorophytes and rhodophytes are primary plastids and are surrounded by one double chloroplast membrane. Dinophytes and chromophytes have undergone secondary and/or tertiary endosymbiotic events and have three or four membranes around the chloroplast. Presumably because of such secondary and tertiary endosymbiotic events, pigment

types have not proven useful as phylogenetic indicators. However, comparisons of gene sequences of the LHC apoproteins indicate two phylogenetically related clusters. One is the 'green-line', with Chl-*a/b*-containing LHCs, and includes green algae, euglenoids and higher plants. Another is the 'red-line' including the red algae, and the Chl-*a/c*-containing cryptomonads, diatoms, brown algae, and dinoflagellates. Such relatedness is also supported by comparisons of other types of gene sequences.

## Further Reading

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