Light Reaction Path of Photosynthesis

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With 118 Figures

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Chapter 2
Phycobiliproteins: Molecular Aspects of Photosynthetic Antenna System

Hugo Scheer[1]

1 Introduction

Harvesting the sun requires both the absorption of the dilute energy, light, and its transformation into chemical energy. With one exception (e. g., the halobacteria), the organisms capable of photosynthesis have these two functions also physically divided. Antenna systems collect the light and guide the excitation energy to the reaction centers, where it is transformed into electrochemical energy. The reaction centers are the conservative part of the photosynthetic apparatus, whereas the size, organization and composition of the antenna varies widely as a developmental and often also individual response to the environmental light quality (Table 1). The reaction centers are always integral parts of the photosynthetic membranes. The antenna may be part of the membrane, too, but it may also be attached on either its inner or outer surface, or even in separate particles or vesicles.

Irrespective of their location, the function of all antenna systems is to store excitation as a temporary buffer, and at the same time guide it to the reaction centers. Depending on the sign and magnitude of the energy gap, as well as on the distance between the antenna chromophores and the reaction center, either of these function is more strongly expressed, or at least more obvious.

The subject of this article may be properly described as a funnel for collecting and feeding excitation energy into the reaction center. It is the biliprotein antenna systems of cyanobacteria and red algae, and of the cryptophytan algae. These pigments are only loosely attached to the photosynthetic membrane and water-soluble, which greatly facilitated their investigation and made especially the former two the hitherto probably best understood antennas on a molecular basis.

This review is focused on the molecular aspects of the process. For recent reviews on biliproteins written from various points of view and citing earlier literature, the reader is referred to the articles of: Bennett and Siegelman (1979), Berns (1971), Bogorad (1975), Chapman (1973), Gantt (1975, 1979), Glazer (1977), MacColl and Berns (1979), O'Carra and O'hEocha (1976), Rüdiger (1971, 1975, 1978, 1979), Scheer (1978, 1981), and Troxler (1975). Biliproteins containing structurally very similar chromophores, the phytochromes and phycochromes, are also involved as reaction center pigments in sensory transduction of green plants and many algae. For recent surveys on these subjects, see: Björn (1979), Hartmann and Haupt (1978), Lazaroff (1973),

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Table 1. Photosynthetic apparatus concerned with the light reactions in various organisms

	Organism type	Reaction centers Major pigments	Location	Antenna Major pigments	Location	Leading references
Anoxygenic photosynthesis	Photosynthetic bacteria (Nonsulfur and purple sulfur)	4 Bphe *a* or *b* 2 Bphe *a* or *b*	PS-Membrane (integral proteins)	Bchl *a* or *b*	PS-Membrane	Clayton and Sistrom (1978)
	Photosynthetic bacteria (Green sulfur)	4 Bphe *a* or *b* 2 Bphe *a* or *b*	PS-Membrane (integral proteins)	Bchl *c*, *d*, *e*	Chlorosomes (separate membrane-covered vesicles)	Clayton and Sistrom (1978)
Oxygenic photosynthesis	Cyanobacteria	1 or 2 Chl *a* (PSI) 1 or 2 Chl *a* (PSII)	PS-Membrane (integral proteins)	Chl *a* (PSI) Biliproteins (PSII)	Phycobilisomes (separate particles at the membrane)	this chapter
	Prochloron	1 or 2 Chl *a* (PSI) 1 or 2 Chl *a* (PSII)	PS-Membrane (integral proteins)	Chl *a*, Chl *b*	PS-membrane	Giddings et al. (1980)
	Red algae	1 or 2 Chl *a* (PSI) 1 or 2 Chl *a* (PSII)	PS-Membrane (integral proteins)	Chl *a*, biliproteins	Phycobilisomes	Giddings et al. (1980)
	Cryptophytan	1 or 2 Chl *a* (PSI) 1 or 2 Chl *a* (PSII)	PS-Membrane (integral proteins)	Chl *a*, *c*, Biliproteins	Intrathylacoidal space	Gantt (1979)
	Brown algae Diatoms	1 or 2 Chl *a* (PSI) 1 or 2 Chl *a* (PSII)	PS-Membrane (integral proteins)	Chl *a*, *c*, carotenoids	PS-membrane	Alberte et al. (1981)
	Dinoflagellates	1 or 2 Chl *a* (PSI) 1 or 2 Chl *a* (PSII)	PS-Membrane (integral proteins)	Chl *a*, *c*, carotenoids	PS-membrane	Song et al. (1977)
	Green algae	1 or 2 Chl *a* (PSI) 1 or 2 Chl *a* (PSII)	PS-Membrane (integral proteins)	Chl *a*, Chl *b*	PS-membrane	Govindjee (1975)
	Green plants	1 or 2 Chl *a* (PSI) 1 or 2 Chl *a* (PSII)	PS-Membrane (integral proteins	Chl *a*, Chl *b*	PS-membrane	Govindjee (1975)

Marme (1977), Mitrakos and Shropshire (19720' Mohr (1972), Pratt (1978), Rüdiger (1971, 1980), Scheer (1981), Smith (1975), and Smith and Kendrick (1976).

2 Morphology

In electron micrographs of the unicellular red algae, *Porphyridium cruentum*, Gantt and Conti described in 1965 a new particle of oblong shape ($\approx$ 4 nm diameter), which was arranged in a rather regular fashion on the outside of the thylakoid membrane. Subsequent investigations by several research groups (Gantt 1979; Glazer et al. 1979; Koller et al. 1978; Wildman and Bowen 1974) revealed similar particles, although of varying size and arrangement, as a main characteristic of the photosynthetic apparatus of cyanobacteria and red algae.

Both classes of photosynthetic organisms owe their coloration to biliproteins, which had been shown by bichromatic action spectroscopy to be major light-harvesting pigments, feeding excitation energy mainly to photosystem II (Emerson 1958; Haxo 1960). The early suspicion that the phycobiliproteins are contained in these particles was confirmed after their isolation as integral entities and the analysis of their composition (Bryant et al. 1976; Gantt and Lipschultz 1974; Gantt et al. 1979; Glazer et al. 1979; Gray and Gantt 1975; Koller et al. 1978; Rigbi et al. 1980; Wanner and Köst 1980). They are almost entirely (Koller et al. 1978; Tandeau de Marsac and Cohen-Buzire 1977; Yamanaka et al. 1978) composed of phycobiliproteins, and are thus properly termed phycobilisomes (Gantt and Conti 1966).

The phycobilisomes contain three different types of biliproteins[2], the phycoerythrins (PE) absorbing in the range between 480 and 580 nm, the phycocyanins (PC) absorbing in the range between 570 and 630 nm, and the allophycocyanins (APC) absorbing in the range between 610 and 670 nm (Table 2). In addition, minor amounts of uncolored proteins have been reported by several workers (Koller et al. 1978; Tandeau de Marsac and Cohen-Bazire 1977; Yamanaka et al. 1978). The majority of the pigment content is PE and PC. The ratio between the two is variable within different species, and in spite of many exceptions, the blue PC's are predominant in the cyanobacteria ("blue algae"), and the red PE's are predominant in the red algae. The ratio between the two is often also variable within a given species in response to the environmental light quality, the relative proportion of the PC's absorbing red light being higher in red and lower in green light (see "chromatic adaptation"). The sizes and fine structures of the phycobilisomes vary accordingly, although a common construction principle is currently evolving.

The detailed investigation of the phycobilisomes revealed a striking morphology. From dissociation experiments, Gantt and co-workers arrived at a model for *P. cruentum* phycobilisomes in which an APC core in contact with the photosynthetic membrane is covered by a roughly hemispherical layer of PC, which in turn is covered by

2 *Abbreviations:* PC = Phycocyanin, PE = Phycoerythrin, APC = Allophycocyanin. The prefixes C–, R– and B– stand for *C*yanobacteria, *r*ed algae and *b*angiales, an order of the red algae. Chl = Chlorophyll

Table 2. Properties of phycobiliproteins and classification according to occurrence, spectra, and subunit composition

Type[a]	Occurence	λmax (nm) in the visible spectral range	Chromophores		
			α–	β–	γ–Chain
APC–I	Cyanobacteria and red algae	656	1x*1a*	1x*1a*	?
– II, – III	Cyanobacteria and red algae	650	1x*1a*	1x*1a*	–
– B	Cyanobacteria and red algae	670	1x*1a*	1x*1a*	–
C–PC	Cyanobacteria	635[d],620,(590)[e]	1x*1a*	2x*1a*	–
PEC[b]	Cyanobacteria	590,568	1xPXB[g]	2x*1a*	–
R–PC	Red algae	620,555	1x*1a*	1x*1a*,1x*2*	–
Cr–PC	Cryptophytan algae	645,610 580 (and others)	1x*1a*[h] (and others)	1x*1a*, 1x*2* (?)	–
C–PE	Cyanobacteria	575[d],560,540	2x*2*	3–4x*2*	–
R–PE[c]	Red algae	568,540,498[g]	2x*2*	? x*2* ? xPUB	? xPUB[j] ? x*2*
b–PE	Red algae	575[d],565,540	2x*2*	4x*2*	–
B–PE	Red algae[j]	565,545,498[f]	2x*2*	4x*2*	2xPUB[j] 2x*2*
Cr–PE	Cryptophytan algae	545–565	? x*2*	? x*2*	–

a Prefixes according to their occurence: B = Brangiales, an order of the red algae; Cr = Cryptophytan algae; C = Cyanobacteria, R = Red algae

b Phycoerythrocyanin

c R–PE has been reported to be a glycochromoprotein (Chapman 1973; Raftery and O'hEocha 1965)

d Possibly an aggregate form (Brown et al. 1975; Zilinskas et al. 1978)

e Shoulder, resolved at low temperatures (Frackowiak et al. 1975; Friedrich et al. 1981, Gray and Gantt 1975; Scheer and Kufer 1977)

Protein structure		Size (kDalton)			
Monomers	aggregation number	α	β	γ	References
$\alpha_3\beta_3\gamma$	1	18	18	30	Gantt and Canaani (1980); Gysi and Zuber (1976); Zilinskas et al. (1978)
$\alpha\beta$	(1),3,6	16	18	–	Bennett and Bogorad (1971); Bogorad (1975); Brown et al. (1975); Cohen–Bazire et al. (1977); Gysi and Zuber (1974); Zilinkas et al. (1978)
$\alpha\beta$	(1),3,6	16	20	–	Glazer and Bryant (1975); Ley et al. (1977)
$\alpha\beta$	1, 3,6	16	20	–	See reviews cited on p. 7
$\alpha\beta$	3	17	20	–	Bryant et al. (1978)
$\alpha\beta$	3,6	18	20	–	Chapman et al. (1967); Gantt and Lipschultz (1974); Glazer and Hixson (1975)
$\alpha\alpha'\beta_2$	1	9,10	16	–	Glazer and Cohen-Bazire (1975); Jung et al. (1980); Mörschel and Wehrmeyer (1975)
$\alpha\beta$	1, 3,6	17	21	–	See reviews cited on p. 7
$\alpha_6\beta_6\gamma$	1	19	19	35	O'Carra (1970); O'Carra and O'hEocha (1976)
$\alpha\beta$	3	19	19	–	Gantt and Lipschultz (1974); O'Carra (1970)
$\alpha_6\beta_6\gamma$	1	19	19	35	Abad-Zapatero et al. (1977); Gantt and Lipschultz (1974); Glazer and Hixson (1977); Sweet et al. (1977); van der Velde (1973)
$\alpha\beta$	2	10	17	–	Glazer et al. (1971); MacColl and Berns (1979); MacColl et al. (1976); Mörschel and Wehrmeyer (1977)

f Shoulder, due to phycourobilin chromophores

g Chromophore of unknown structure type (λ_{max} = 600 nm after denaturation in 8 M urea, pH 3.0)

h In addition, chromophores of unknown structure have been described (λ_{max} = 690 nm after denaturation in 8 M urea, pH 2.0)

i Chromophore of the urobilin spectral type. The exact structure is unknown (λ_{max} = 498 nm after denaturation in 8 M urea, 1 M HCl)

j A pigment spectroscopically similar to B–PE has been described to occur in a marine cyanobacterium (Shimura and Fujita 1975)

PE. Phycobilisomes can be isolated intact in high ionic strength buffer both with and without parts of the thylacoid membrane still attached, and the dissociation has been studied both by fluorescence spectroscopy (Gantt and Zilinskas 1976; see below) and immunoelectron microscopy (Gantt and Zilinskas 1978; Gantt and Lipschultz 1977). The phycobilisomes of *P. cruentum* are rather large. Indications of a fine structure have been obtained only recently, including a small stalk which may function as an anchor to the membrane (Wanner and Köst 1980).

The sequential arrangement of the pigments, APC, PC, and PE, has been supported by the analysis of phycobilisomes from another red alga, *Rhodella violacea*, by the group of Wehrmeyer (Koller et al. 1978; Mörschel et al. 1977). Due to their smaller size and an almost planar shape it has been possible to get a more detailed insight into their morphology, which is schematically shown in Fig. 1. The inner core of the phycobilisome consists of three double-platelets of APC, arranged in a triagular fashion and attached edge on to the thylacoid membrane. This core is "garnished" with short rods, termed tripartite units due to their composition of three double-platelets stacked face to face like a stack of coins. The inner double-platelet facing the APC core is an aggregate of PC, the outer two are composed of PE. The individual APC and PC platelets are trimers of the α, β - monomers of these pigments (see below) having a ring-shaped structure with an inner hole (Mörschel et al. 1980). The double-platelets then are hexamers. The PE present in *R. violacea* is a B-type pigment (Table 2), in which the inner hole of the hexamer contains an additional polypeptide chain, thus making it an $\alpha_6\beta_6\gamma$ complex (Abad-Zapatero et al. 1977; Köst 1980; Sweet et al. 1977; Wehrmeyer 1980).

Both trimers and hexamers have been shown by in-vitro studies to be the aggregates formed predominantly and reversibly from manomers in solution (Berns and Morgenstern 1968; Chen and Berns 1978; Lee and Berns 1968; MacColl and Berns 1973; MacColl et al. 1971 a, b, 1974; Saito et al. 1976). The same or similar aggregates

have also been suggested as the building blocs of phycobiliprotein crystals (Bryant et al. 1976; Dobler et al. 1972; Sweet et al. 1977). Some apparently confliction results between morphological, biochemical and biophysical investigations on biliprotein aggregates have been discussed recently Mörschel et al. (1980).

The phycobilisomes of cyanobacteria have been characterized in comparable detail only recently, due to isolation problems (Gantt et al. 1979; Gray and Gantt 1975; Gray et al. 1973; Rigbi et al. 1980; Yamanaka et al. 1978). Electron microscopy of three species has yielded basically the same fine structure, with the number of the central APC platelets or the lenght of the rods being somewhat variable (Glazer et al. 1979).

The phycobilisomes are probably products of a complex self-assembly process which does not only require the aggregation of identical biliproteins (homo-aggregation), but also of different types of biliproteins with each other (hetero-aggregation) and with the membrane, in a highly ordered and regulated fashion. The organizing principles have only very recently begun to emerge. Homo-aggregates larger than the hexamer and heteroaggregates have been isolated by several groups from partly dissociated phycobilisomes (Kessel et al. 1973; Koller et al. 1978; Mörschel et al. 1977, 1980a, b; Yamanaka et al. 1978; Gantt et al. 1979; Rigbi et al. 1980; Grabowski et al. 1981), but their reassociation was rarely observed and difficult to achieve in a reproducible way.

After the identification of colorless proteins as integral components of phycobilisomes, at least some of them have tentatively been related to an ordering function (Tandeau de Marsac and Cohen-Bazire 1977b). An indirect evidence to this is the finding, that crude dissociates of phycobilisomes containing larger fragments still bearing colorless proteins can be reassociated into functional phycobilisomes (Canaani et al. 1980; Katoh, private communication). The largest one of the colorless proteins (75–90 kDalton) is probably involved in the attachment of phycobilisomes to the photosynthetic membrane (see Gantt 1981). A protein of this size has recently been identified both in isolated phycobilisomes and in membranes from which the biliproteins had been dissociated (Redlinger and Gantt 1980). This protein is blue, however, with the fluorescence characteristics of an APC (–B?), and its relation to the colorless proteins is still unresolved (Gantt 1981). Irrespective of its coloration, an "anchor" protein has also been suggested from electron-micrographs of phycobilisomes from *P. cruentum* showing a footlike protrusion (Wanner and Köst 1980).

Three different smaller colorless proteins (nominally 33, 30 and 27 kDalton) have recently been isolated from *Synechococcus* 6301 phycobilisomes (Lundell et al. 1981). From reassociation experiments with isolated phycocyanin and one or more of the rather hydrophobic colorless proteins, specific functions for the latter have been suggested. The 33 and 30 kDalton species are involved in stacking hexamers into rods, which may grow to exceptional and unnatural lengths. The 27 kDalton protein is rather related to rod termination. Only small stacks are formed in its presence, even if the 30 and/or 33 kDalton proteins are present as well. A small colorless protein has also been found in PC–PE complexes isolated from *P. sordidum* phycobilisomes, where it appears to function as a linker between the different pigments (Lipschultz and Gantt 1981).

These findings do assign specific functions to at least some of the colorless proteins (group II). As an intriguing aspect of these results, one can imagine, that the self-assembly of phycobilisomes is controlled by the relative concentrations of the different biliproteins *and* colorless proteins, which in turn is controlled by their biosynthesis and degradation.

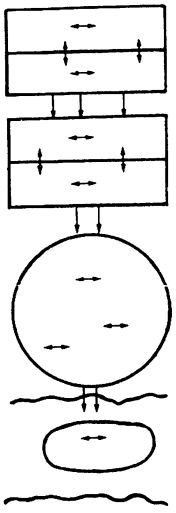
In many cyanobacteria, the amounts of the different biliproteins (and other photosynthesis pigments) are regulated by light (chromatic adaption, see below and Bogorad 1975), by nitrogen supply (Allen and Smith 1969) and sulfur compounds in the medium (Schmidt 1980).

On the phycobilisome level, these regulations have been shown to involve the composition of the colorless proteins (Tandeau de Marsac and Cohen-Bazire 1977b; Yamanaka and Glazer 1980), as well as the composition of the biliproteins and the phycobilisome-architecture (Siegelman 1980; Yamanaka and Glazer 1980). For biophysical studies, the contamination of biliproteins with colorless proteins in amounts varying with the isolation procedure, has to be considered as a potential complication, which e.g. may be involved in the conflicting results on size and shape of biliprotein homoaggregates.

The morphology of cyptophytan antennas is different from those of cyanobacteria and red algae. Phycobilisomes are absent, and the biliproteins rather appear to be locadet at the inner side of the thylacoid membrane (Gantt et al. 1971; Wehrmeyer 1970). The grana lamellae are wider spaced than in green plants, and filled with an electron-dense material assigned to biliproteins, for which only recently indications of a fine structure have been obtained (Wehrmeyer, pers. comm., 1979). For a recent survey on cryptophytan biliproteins, see Gantt 1979).

3 Energy Transfer

The sequential arrangement of pigments in the phycobilisomes from PE to PC to APC and further to the membrane containing chlorophyll *a* is in the proper order for a downhill energy transfer (Fig. 2), which is indeed their major function. An transfer efficiency of close to 100% from PE to chlorophyll *a* has been determined in intact algae, and



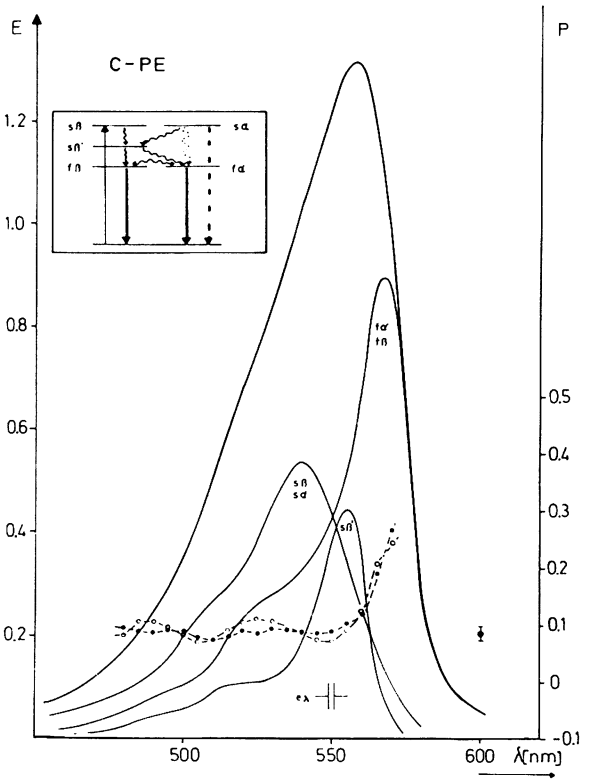


Fig. 3. Deconcolution of the long-wavelenth absorption band and the corresponding terms scheme of C–PE from *Pseudoanobaena* W 1173, according to Zickendraht et al. (1980). The deconvolution is based on absorption, fluorescence polarization data of the isolated α- and β-subunits, and of the $\alpha\beta$-monomers, as well as on an independent computer deconvolution of the absorption spectra The *open* and *full circles* are fluorescence polarization data (scale on the right)

from PE to APC in isolated phycobilisomes (Porter et al. 1978; Searle et al. 1978). This efficient energy transfer can be traced down to the subunits of the individual pigments. All biliproteins are highly fluorescent in their native state (see below), but their emission spectra are not symmetric to their long-wavelength absorption bands, and they have a low and, except for APC, varying degree of fluorescence polarization (Dale and Teale 1970; Grabowski and Gantt 1978a, b; Teale and Dale 1970; Zickendraht-Wendelstadt et al. 1980). Both results have been interpreted first by Teale and Dale (1970) and subsequently by others in terms of distinct and different chromophores in the biliproteins, with an efficient energy transfer between the chromophores, and only the ones lowest in energy fluorescing. On this basis, it is possible to deconcolute the absorption spectra of biliproteins and even their subunits into two (or more) components, arising from the fluorescing "f" and the sensitizing "s" chromophores, respectively (Fig. 3). This concept of different chromophores has later been supported by chemical evidence (see "the protein").

However, a distinction between "s" and "f" chromophores is purely phenomenological, since it is not a property of a chromophore per se, but rather of the aggregation state of the pigments. In all biliproteins and their functional aggregates, it is always the chromophores lowest in energy which fluoresce exclusively or at least predominantly. Biliproteins have 1 to 4 chromophores on the individual subunits, up to 40 chromophores in the monomer superstructures of B– or R –PE, or the APC, PC and PE hexamer build-

ing blocs of phycobilisomes, and between 900 and 2500 chromophores in a *P. cruentum* phycobilisome, as estimated from the data of Wanner and Köst (1980) and Gantt (1976)[3]. In building up the latter, the percentage of the "f" chromophores is constantly decreasing. Thus, the "f" chromophore of isolated PE becomes an "s" chromophore in PE–PC heteroaggregates (Koller et al. 1978; Grabowski et al. 1980), and in the phycobilisomes it is only the few APC chromophores which fluorescence (Grabowski and Gantt 1978a,b; Porter et al. 1978; Searle et al. 1978).

The energy transfer of the individual subunits, the monomers and the various aggregates of the pigments has been analyzed in terms of a weak coupling (Förster) process. From the absorption and fluorescence spectra critical distances for non ordered orientations have been calculated (see Grabowski and Gantt, 1978 for further references), which are considerably larger than the diameters of the subunits, allowing an efficient transfer. The spatial distribution of the chromophores in different isolated phycobiliproteins has been estimated based on the Förster mechanism and the Jablonski "active shere" approximation. They indicated a surface distribution (Dale and Teale 1970; Zickendraht et al. 1980), in agreement with chemical evidence (see below). The observed energy transfer times within individual pigments (Kobayashi et al. 1979) and phycobilisomes (Searle et al. 1978) are also cinsistent with and analyzed on the basis of this transfer mechanism. For phycobilisomes of *P. cruentum*, hopping times of 280 ± 40 ps, with an average of 28 jumps for the transfer from PE to PC, have been determined in agreement with the model described, when treated by the *Pearlstein* formalism (Grabowski and Gantt 1978b). The energy transfer has been investigated, too, for the individual subunits of a C–PE containing two and three chromophores, respectively, and the relative orientations of the dipoles determined (Zickendraht et al. 1980). The term scheme shown in Fig. 3 has been obtained from this work.

Strong coupling between chromophores seems to be less prominent in phycobiliproteins from cyanobacteria and red algae, and has in no case yet been shown conclusively. From CD data, exciton coupling has been implied for an APC (Gantt and Canaani 1980). The S-shaped CD bands for C–PC from *Pseudoanabaena spec.* W 1173 are indicative, too, of exciton splitting, but here a definite decision is difficult in view of the five different chromophores present (Langer et al. 1980).

An intermediate coupling, has finally been suggested in APC to account for the pronounced red-shift upon trimer formation without an accompanying CD-effect (McColl et al. 1980). In view of the increase of the oscillator-strenght of the long-wavelength band, a chemical change (conformation, protonation) may be considered as well.

By contrast, strong coupling between chromophores has been deduced mainly from CD data for a cryptophytan PC (Jung et al. 1980). This is supported by a fast component ($\leqslant 8$ ps) in the transient absorption of this pigment, assigned to an energy transfer process, as compared to 84 ps in a C–PC monomer (Kobayashi et al. 1979). This indicates again a certain separation of the cryptophytan biliproteins, which is further evidenced by their spectral diversity and the occurence of special chromophores (see below, and Gantt 1979).

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3 The "monomers" of B–PE, R–PE and possibly one of the APC have the rather complex $\alpha_6\beta_6\gamma$-structure, in which the γ- subunit fills the inner hole of the torus-shaped trimers and hexamers typical for most biliproteins (see below)

From the morphological and energy transfer studies two different strategies for harvesting green and orange light, their conversion to excitation energy corresponding to red light quanta, and their funneling to the reaction centers, seem to have been followed during the evolution of biliprotein antennas. One is exemplified in the cyanobacteria, where each of the pigments present covers only a comparably narrow absorption range. To avoid gaps in the transfer chain and the absorption spectrum, the phycobiliproteins are arranged in intricate superstructures, the phycobilisomes. In the cryptophytan biliproteins, on the other hand, a miniature transfer chain has already evolved within each biliprotein, especially within the phycocyanins. Accordingly, a superstructure is not necessary, although the transfer from PE to Chl *a* would be facilitated by the Chl *c*'s present in these organisms as intermediate carriers. In the red algae, both concepts have been united, since they have biliproteins covering a broader range of energies organized in phycobilisomes.

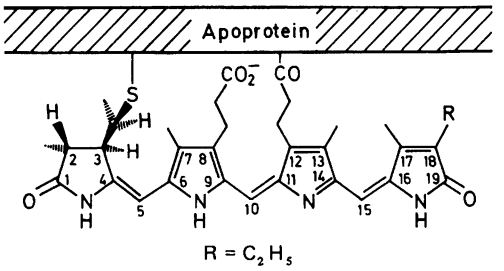
4 Chromophore Structure

4.1 Chromophores Cleaved from Biliproteins

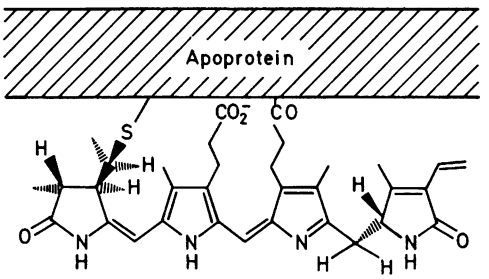
Only two chromophores are responsible for the broad range of absorption spectra of the majority of biliproteins (Table 2). Both are of the formerly[4] so-called IX α substitution type, characteristic for the mammalian bile pigments derived from heme cleavage at the methine bridge formerly designated "α", now C–5. Phycocyanobilin (*1*) is the blue chromophore of PC's and APC's. It is noteworthy that the same chromophore apears to be present in the phycochromes (Björn 1979; Ohad et al. 1979; Ohki and Fujita 1978; Scheibe 1972), and a very similar chromophore (18-vinyl instead of 18-ethyl) occurs in phytochrome(s) in the P_r - form (Grombein et al. 1975; Rüdiger 1980). Phycoerythrobilin (*2*) is the red chromophore of PE's. Some of the biliproteins contain additional chromophores. R– and B–PE's carry phycourobilin chromophores, for which structure *3* has been proposed (O'Carra and O'hEocha 1976). Phycoerythrocyanin has a red chromophore of unknown structure (Bryant et al. 1978), and at least two other chromophores have been proposed to occur in cryptophytan biliproteins (Glazer and Cohen-Bazire 1975; Jung et al. 1980; Mörschel and Wehrmeyer 1975). The evidence for these less-known chromophores comes from spectroscopic studies on the denatured pigments, which is useful for screening. If done under carefully controled conditions, chromophores other than *1* or *2* can be easily recognized. It should be pointed out, however, that the structures *1* and *2* have been strictly proven only for a few biliproteins, and that alterations, e.g., of the side chains or especially the second protein bond, may remain unnoticed in such studies.

All chromophores are covalently bound to their respective apoproteins. This prevented a direct examination by the common analytical tools with the exception of uv-vis spectroscopic technics, and required initial degradative steps. Chromophore cleavage,

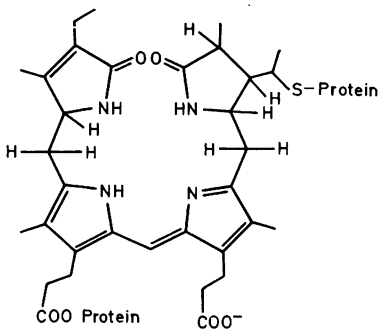
4 A new IUPAC nomenclature of bile pigments has recently been agreed on (IUPAC 1979). For a survey of the older nomenclature systems, see Bonnett (1978)



Structure 1



Structure 2

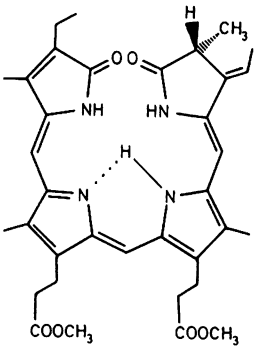


Structure 3

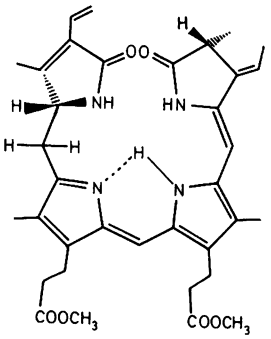
chromic acid degradation or proteolytic digestion of the peptide chains have been most useful. Recently, milder degradative techniques have been investigated, too.

The chromophore cleavage reactions yield different pigments depending on the method used. The temporary confusion concerning the nomenclature of the chromophores is mainly due to the different cleavage procedures yielding different products. For a discussion, the reader is referred to O'Carra (1980), Rüdoger (1971, 1975), and to the new IUPAC nomenclature on tetrapyrroles (1979). Most studies have focused on the 3-ethylidene bilins *4* and *5*, which can best be obtained by thermolysis in refluxing alcohols (Fujita and Hattori 1961; O'Carra and O'hEocha 1966; Fu et al. 1979), or by treatment with hydrogen bromide in trifluoracetic acid (Schram and Kroes 1971). The same products have been obtained recently by a flash-pyrolytic procedure (Fu et al. 1979). The structures *4* and *5* have been elucidated by chromic acid degradation (Rüdiger 1969) yielding succin- or maleimides carrying at least in principle the substituents of the parent pyrrolic rings (Rüdiger and O'Carra 1969; Rüdiger et al. 1967), and by ^{1}Hmr and and mass spectroscopy (Chapman et al. 1967; Cole et al. 1968; Crespi et

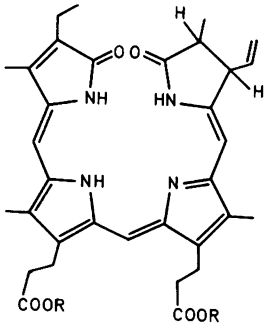
Structure 4



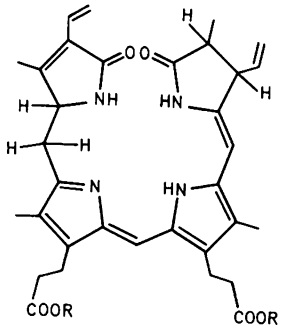
Structure 5



Structure 6



Structure 7



al. 1967, 1968). Both have been confirmed by total synthesis (Gossauer and Hinze 1978; Gossauer and Hirsch 1974; Gossauer and Weller 1978).

Cleavage in aqueous acid yields different pigments, to which structures *6* and *7* have tentatively been assigned. They contain the same conjugated system as do *1* and *2*, respectively, but the exact nature especially of the substitutent at C–3 has not yet been established. One of the possible products, the epimeric methanol adducts at $C-3^1$, has recently been synthesized (Gossauer et al. 1981). Products of this type probably derived from *4*, together with *4* itself, are excreted into the medium by a mutant of the blue-colored unicellular red algae, *Cyanidium caldarium* (Troxler et al. 1975; Beuhler et al. 1976).

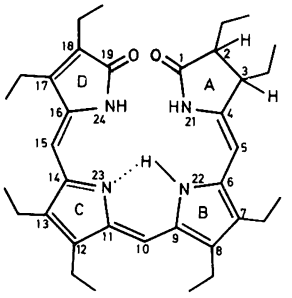
The R configuration of *4* and *5* at the asymmetric C–2 has been arrived at by chromic acid degradation to an imide of known stereochemistry (Brockmann and Knobloch 1973), the 16R configuration by asymmetric synthesis (Gossauer and Weller 1978), and correlation with 4R, 16R urobilin (Cole et al. 1967). The cleavage of biliproteins to *4* (and probably *5* as well) yields a mixture of the Z and E isomers of the 3-ethylidene substituent, as shown by total synthesis (Weller and Gossauer 1980).

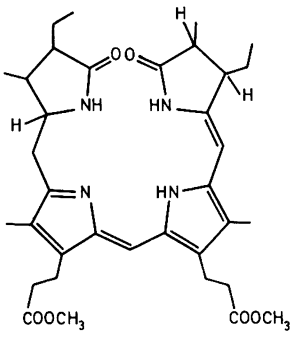
4.2 Chromophores Bound to the Protein

The conjugation systems of the chromophores still bound to the proteins have been investigated mainly by uv-vis spectroscopy. Although the spectra of biliproteins are

strongly influenced by chromophore-protein interactions (see below), these can be completely abolished by unfolding the protein, e.g., by 8 M urea or guanidinium chloride (Köst et al. 1975; Kufer and Scheer, 1979; see there also for leading references to earlier work). Heat denaturation leads to complete uncoupling of the chromophores as well (Scheer and Kufer 1977), whereas mercurials (Pecci and Fujimori 1967, 1968; Erokhina and Krasnovskii 1968), pH changes (Erokhina and Kransnovskii 1968; Zickendraht-Wendelstadt 1980) and sodium dodecylsulfate give only partially uncoupled and/or further modified chromophores. The denatured pigments can be directly correlated to free bile pigments of known structure not only as the free bases, but also as the anions, zinc complexes and, preferably, the cations, the latter being most stable and having more intense and sharp absorption bands. By acid-base titration, the pK values for protonation and deprotonations are accessible as additional criteria (Grombein et al. 1975). By this means, *1* has been shown to have the same conjugation system as does *8* (Kufer and Scheer 1979), and *2* to have the conjugation system of *9* (Köst et al. 1975). The position of the long-wavelength maxima of bile pigments in the visible absorption range is roughly proportional to the length of the conjugation system, which is helpful in the investigation of the less-known chromophores (Köst et al. 1975).

Structure 8





Structure 9

All bile pigment chromophores of phycobiliproteins are covalently bound to the proteins. A thioether bond joining ring A to a cystein residue has now been established as a characteristic feature of the biliproteins (formulas *1, 2*). This type of bond had already been proposed by *Fujiwara* in 1956 from the amino acid analysis of chromopeptides. Cystein as the binding amino acid could first be established for a peptide derived from B–PE of *Porphyridium cruentum* (Köst-Reyes at al. 1975). It has since been recognized as the binding amino acid for *all* chromophores in several PC's and PE's from sequencing studies (Brooks and Chapman 1971; Bryant et al. 1976; Byfield and Zuber 1972; Crespi and Smith 1970; Frank et al. 1978; Freidenreich et al. 1978; Harris and Berns 1975; Kililea and O'Carra 1968; Köst-Reyes and Köst 1979; Lagarias et al. 1979; Muckle et al. 1978; Troxler 1980; Williams et al. 1978; Zuber 1978) and a comparison of the number of free and bound SH residues (Glazer et al. 1979).

The chromophores are attached to the cystein residues via the 3-ethyl substituents as shown in formulas *1, 2* and *3*. Both the site of attachment at C–3^1 and the stereochemistry have been deduced from a modification of the chromic acid degradation and model studies on succinic imides, and from recent ^{1}Hmr investigations of bilipeptides.

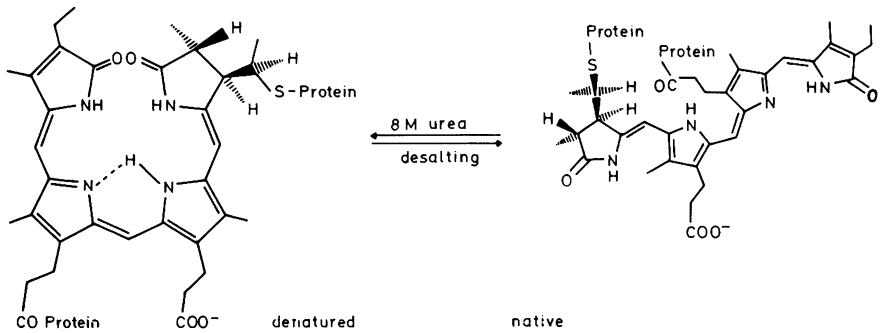
Chromic acid degradation at ambient temperature leaves ring A attached to the protein, but the thioether is oxidized to a sulfone under the reaction conditions (Schoch et al. 1976). The latter can subsequently be eliminated unter very mild conditions with ammonia to yield ethylidene succinimide *5* (Klein et al. 1977). This sequence is stereospecific and allowed (in conjunction with the known 2R configuration (Brockmann and Knobloch 1973) the determination of the 2R, 3R, 3^1R configurations of the three asymmetric C atoms at ring A (Klein and Rüdiger 1978; Lotter et al. 1977).

The thermal (Fujita and Hattori 1962; O'Carra and O'hEocha 1966; Fu et al. 1979) or acid/HBr catalyzed (Schram and Kroes 1971) cleavage of the chromophores from the protein proceeds by a similar mechanism to yield the well-known ethylidene bilins *4* and *5* from *1* and *2*, respectively. Under the reaction conditions, epimerization at C–2 (Gossauer and Weller 1978; Scheer and Bubenzer 1978) and isomerization of the $3,3^1$ - ethylidene substituent (Weller and Gossauer 1980) is possible. The 3-vinyl bilins *6* and *7* suggested as products from the acidic cleavage, can be rationalized by a different elimination mechanism (O'Carra et al. 1980). Other cleavage products may arise from further reactions of the 3-ethyldene, 3-vinyl and 18-vinyl groups (Brandlmeier et al. 1979; Klein and Rüdiger 1979; Weller and Gossauer 1980). Independent proof for the structure *1* and thioether bond in both PC from *Synechococcus* sp. 6701 and Phytochrom P_r comes from ^{1}Hmr spectroscopy of bilipeptides. Both yield very similar signals for the chromophore, with the exception of the ones originating from the C–18 substituent. In particular, the analysis of the signals originating from ring A-substituents support the thioether linkage (Lagarias et al. 1979; Lagarias and Rapoport 1980). In an extension of the studies on only one of the three different chromophore-regions (Lagarias et al. 1979), the remaining two have recently been investigated (Lagarias 1981). Surprisingly, one of them (β_2) appears to be differently bound, still via cystein, but possibly to ring D.

The evidence for additional protein-chromophore bonds is much less sound than for the thioether bridge. Chromic acid degradation has yielded conflicting results on the presence of a second protein bond to the propionic acid side chain at ring C (or B), involving most probably a serin residue (Brooks and Chapman 1972; O'Carra and O'hEocha 1976; Rüdiger 1971, 1975, 1979). Only one peptide containing such a bond has so far been identified (Muckle et al. 1978), indicating that in a given protein only part of the chromophores may contain a second bond of this type. Other additional bonds discussed are a ring A lactimester-aspartic acid junction (Crespi and Smith 1970), and a bond to glutamate (Brooks and Chapman 1972). No indications for such additional bonds have been obtained from ^{1}Hmr-studies of bilipeptides (Lagarias 1981; Lagarias et al. 1979). The problem in verifying such bonds is their lability as compared with the thioether bond to proteolytic or hydrolytic conditions, which requires less drastic degradation procedures. The chromate oxidation has been applied for this purpose (Rüdiger 1969, 1971, 1975), and recently two procedures cleaving selectively between rings A and B (Krauss and Scheer 1981) and between rings B and C (Kufer and Scheer 1981) have been applied to PC from *Spirulina platensis* (see Scheer, 1981, for discussion). The latter indicate, that bonds may be present in only part of the chromophores.

5 Noncovalent Protein Chromophore Interactions

Native phycobiliproteins are highly fluorescent (see Sect. 3), whereas the pigments denatured by urea or heat fluoresce with very small quantum yields (Kufer 1977; Kufer et al. 1980; Zickendraht-Wendelstadt 1980). Similar pronounced differences are observed in the absorption spectra (Fig. 4; Sect. 4.2) and the chemical reactivities (Table 3) of the native as compared to the denatured pigments. As can be shown, e.g., by SDS gel electrophoresis, the covalent bonds between chromophores and proteins are unchanged by denaturation. Moreover, the spectroscopic properties of the native pig-



Structure 10a Structure 10b

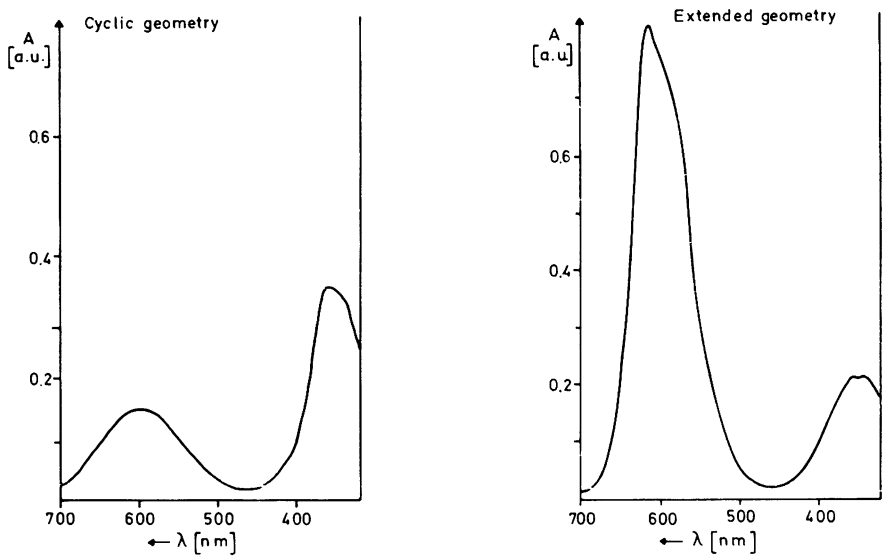


Fig. 4. Absorption spectra of denatured (*left*) and native (*right*) C-PC from *Spirulina platensis*, and the corresponding types of chromophore geometry. Both spectra correspond to identical pigment concentrations. The cyclic conformation is helical (see formula *11*). The extended conformation of the native state is chosen with respect to minimum steric hindrance of the substituents. It is probably uniformly twisted, too, as inferred from CD data (see text)

Table 3. Properties of native biliproteins, as compared to the properties of denatured biliproteins and free bilins

	Denatured biliproteins and free bilins	Native biliproteins	Ecological significance in the native pigments
Absorption spectra	Broad bands Near UV band: $\epsilon \approx 4 \cdot 10^4$ Visible band: $\epsilon \approx 2 \cdot 10^4$ λ_{max} determined by the chromophore only	Narrow bands Near UV band: $\epsilon \approx 2 \cdot 10^4$ Visible band: $\epsilon \approx 10^5$ λ_{max} influenced by the protein	Increased absorption, fine-tuning of λ_{max} However, only narrower spectral range covered by any given chromophore
Photochemistry	Radiationless deactivation predominant, small quantum yield of fluorescence and photochemical reactions	High quantum yield of fluorescence (phycobilins) or photochemical reactions (phytochrome), respectively	Decreased losses of excitation energy
Reactions: Complexation Reduction Oxidation	Instantaneous complexation with Zn^{2+} at ambient temperature Complete reduction with dithionite ($c \geqslant 0.5$ mM) Oxidative bleaching within days at 4 °C	Inert with Zn^{2+} at ambient temperature Partial reduction only with dithionite ($c \geqslant 5$ mM) Stable for months at 4 °C	Chromophore stabilization

ments can be restored fully and in good yield (Scheer and Kufer 1977) within the folding time of the protein (Bartholmes and Scheer 1980) if the concentration of the denaturing agent or the temperature is decreased.

The spectroscopic properties and reactivities of the denatured pigments are identical to those of free bile pigments of the proper structure, which has been commonly used in the structure analysis of biliproteins (see Sect. 4). This proves, on the other hand, that the properties of the denatured pigments can be accounted for completely by their proposed structures, and that the changes upon naturation are entirely due to noncovalent interactions between the chromophores and the proteins, and/or among the chromophores.

The ecological significance of these changes is obvious from Table 3. The oscillator strength, as a base for the efficiency of light absorption of biliprotein antenna systems, is enhanced. Radiationless deexcitation, corresponding to a waste of the absorbed energy, is decreased, and at the same time chemical and photochemical side reactions leading to the destruction of the sensitive bile pigment chromophores is impeded. The biliproteins thus present an excellent example of molecular ecology, e.g., the adaptation of photoreceptor molecule as unfit as an A-dihydrobilindion like *1* or *4*, to its function in an antenna pigment as efficient as phycocyanin. Recently, some progress has been made in understanding the principles involved, by a combination of chemical model studies, MO calculations, and denaturation-renaturation studies with the isolated pigments.

5.1 Topology of the Chromophore

The characteristics of the changes in the absorption spectra can be accounted for essentially by a change in the geometry of the chromophores. Bile pigments are at least principally flexible structures, and several research groups have treated theoretically the dependence of the absorption spectra on the molecular topology (Burke et al. 1971; Chae and Song 1975; Falk and Höllbacher 1978; Fuhrhop et al. 1974; Wagnière and Blauer 1976), and in some calculations also on a charge at or close to the π-system (Pasternak and Wagnière 1979; Sugimoto et al. 1976). The results agree in one fundamental aspect, that the ratio of the oscillator strengths of the two lowest electronic transitions,

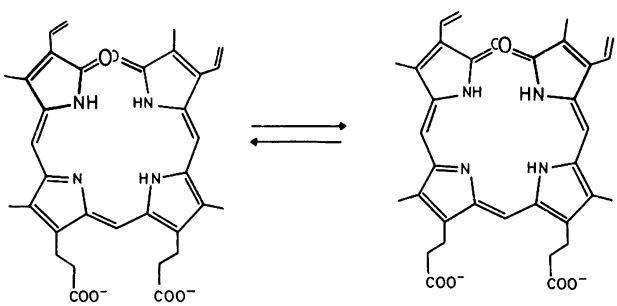
$$Q = \frac{f_{vis}}{f_{near\ uv}}$$

is a rough measure of the shape of the molecule. Q is < 1 in cyclic porphyrin-type conformations, but becomes $\gg 1$ when the molecule is stretched to an extended conformation. On this basis, the spectroscopic properties of native and denatured phycocyanin can be rationalized by the former having an extended *(10b)*, the latter a cyclic conformation *(10a*, Fig. 4) (Scheer and Kufer 1977).

The theoretical calculations have been supported by the conformational analysis of bile pigments of the biliverdin type in solution and in the crystal. A cyclic-helical conformation was proposed first for optically active urobilin on the basis of the large cotton effects observed, which are typical for inherently dissymmetric chromophores (Moscowitz et al. 1964). If the two asymmetric centers at C–4 and C–16 have the same

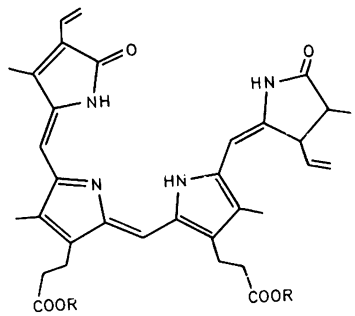
configuration, one of the two helices of opposite chirality is strongly favored due to steric hindrance. The effect of asymmetric centers in shifting the equilibrium between the two helices is principally possible, too, in other helical bilins. For denatured PC, an energy difference of $\Delta H = 0.1-0.2$ kcal/mol has been estimated from the steric hindrance arising from the asymmetric C–2, C–3, and C–3' (Scheer et al. 1979). In denatured PE, the contribution of C–16 is expected to counteract this effect. In agreement with this reasoning, denatured PC (Lehner and Scheer 1981), but not PE (Langer et al. 1980), has a pronounced optical activity.

On the other hand, optical activity can be induced, too, by a chiral environment. The solvent-induced circular dichroism (SICD) seems to be a safe indicator of helical conformations (Lehner et al. 1978, 1981). *All-syn, Z* biliverdin-dimethylester *(11)* gives a strong SICD effect (Lehner et al. 1978), but neither the *anti-E, syn-Z, Syn-Z* isomer *12* (Gossauer et al. 1980) nor the formyltripyrrinone *13* (Lehner et al. 1981) do. In solution *11* has a predominantly helical conformation (Lehner et al. 1978; Falk et al. 1978a), and a helical crystal structure (Lehner et al. 1978b; W.S. Sheldrick 1976). The energy barrier of 42 kJ/mol between the two helical forms allows a rapid interconversion at ambient temperatures (Lehner et al. 1979). In a chiral solvent, e.g., lactate esters, the equilibrium between the two helical forms is shifted. As both forms are expected to be strongly optically active, a slight shift of the equilibrium from K = 1 is already sufficient to give measurable CD signals. In another way of reasoning, the perturbation by the chiral solvent is transmitted through the entire molecule only in a helical structure. *12* has a more extended conformation (according to MO studies), with no interaction between rings A and D (Falk et al. 1978b), while *13* is planar, as has been shown by the X-ray structure of an analog (Cullen et al. 1978), and thus also lacks the principal requirements for a strong SICD-effect. Falk et al. (1981) have recently carried out force field calculations on bile pigments. For the Z, Z, Z-bilindion, they converge at a structure very similar to the crystal structure of biliverdin-dimethylester (Sheldrick 1976).

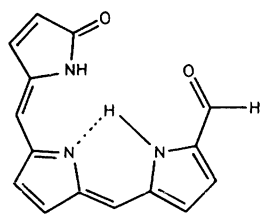


Structure 11a Structure 11b

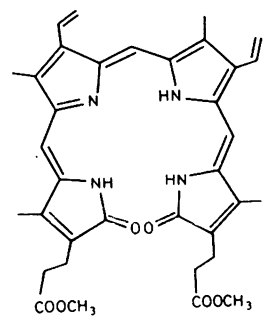
Experimental data on bile-pigments with restricted conformational freedom have been provided from pigments bearing intramolecular bridges. A 21,24-methano-bilindion supports the assignment of cyclic-helical conformations to free bilindiones (Falk and Thirring 1981). Models for the extended conformations suggested for the native biliprotein-chromophores, have been provided by nature and biomimetic synthesis with isophorcabilin (*15*) and related polycyclic pigments (Bois-Choussy and Barbier 1978; Choussy and Barbier 1975). They are derivatives of the 3, 7, 12, 17-tetramethyl-8,13-divinyl-2, 18-dipropionyl-bilindion (*14*, "biliverdin IX γ")(Bois-Choussy and Barbier 1978; Choussy and Barbier 1975), and have been discovered during investigations on bile pigments from caterpillars and butterflies (*Lepidoptera*). Their importance for conformation analysis comes from their confinement to extended conformations by the additional rings, which are formed by intramolecular additions to the vinyl groups of *14*.



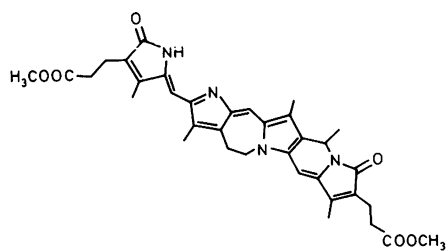
Structure 12



Structure 13



Structure 14



Structure 15

The isophorcabilin *15* has an intense visible absorption band and a very weak near absorption (Bois-Choussy and Barbier 1978; Brandlmeier et al. 1981), thus supporting the theory. Its spectrum is very similar to that of native PC, for which an extended conformation is therefore highly likely, too (Fig. 4) (Scheer and Kufer 1977). This extended conformation has to be brought about by the protein, but the details of the process are not yet understood. It should be pointed out, that extended conformers of bile pigments like *1* and *12* are less stable than their cyclic conformers (Falk and Grubmayr 1979; Scheer et al. 1979). The energy for unfolding the chromophores would then have to be provided by the protein (Kufer and Scheer 1979). In agreement with this reasoning, the energies of denaturation of various PC's determined recently by Chen and Berns (1978) are conspicuously low as compared with the respective energies of globular proteins of similar size (Knapp and Pace 1974; Salahuddin and Tanford 1970).

5.2 Conformational Mobility

Another factor of the protein seems to be a restriction of conformational mobility to the chromophore. Bile pigments of the biliverdin type have broad absorption spectra, which even at low temperatures show only little fine structure (Chae and Song 1976; Friedrich et al. 1981a,b; Gautron et al. 1976; Holzwarth et al. 1978; Petrier et al. 1979, 1980; Scheer and Kufer 1977). This has been rationalized as a superposition of the spectra several conformers with slightly different absorptions, which are in rapid equilibrium with each other (Lehner et al. 1978a; Scheer et al. 1977; Scheer and Kufer 1978). This interpretation has been substantiated recently by a careful fluorescence analysis. It could be shown that a solution of *11* contains at least two species, their equilibrium being solvent-dependent (Braslavsky et al. 1980; Tegmo-Larsson et al. 1980a, b). From the Q values of the excitation spectra, a cyclic and a more extended conformation have been proposed, the latter being favored in rigid solutions and especially in liposomes. Broad spectra are characteristic of most bile pigments, but a notable exception is the "purpurins", e.g., *13*. They have a double-peaked long-wavelength absorption, with each of the two components being narrow. *13* is planar (Cullen et al. 1978), and it has been suggested, that the two peaks arise from two comparably rigid, distinct forms, e.g., two tautomers, each fixed by a different type of intramolecular H-bond (Scheer et al. 1977).

Native biliproteins have broad absorption bands, too, in which some fine structure is generally obvious already at ambient temperature (see for examples: O'Carra and O'hEocha 1976) and becomes prominent at low temperatures (Frackowiak and Grabowski 1971; Frackowiak et al. 1975; Friedrich et al. 1980a,b; Gray et al. 1976; Scheer and Kufer, 1977; Zickendraht-Wendelstadt et al. 1980). Thus, their spectra are superpositions of different forms as well. There is, however, an important difference as compared with the denatured biliproteins or the free bile pigments with corresponding structures: The different forms are not in equilibrium, but rather correspond to different chromophores of the pigments in different environments well defined by the protein (see Scheer 1981; Zickendraht-Wendelstadt et al. 1980; Zuber 1978, for leading references). The situation is reminiscent the purpurins, but obviously for different reasons. As an example, C–PC contains three chromophores. Its long-wavelength absorp-

tion band is asymmetric with a distinct shoulder at shorter wavelength already noticeable at room temperature, which is split into two narrow components at low temperatures (Frackowiak et al. 1975; Friedrich et al. 1980a, b; Scheer and Kufer 1977). The assignment to individual chromophores is yet unclear. The renatured α– and β-subunits of C–PC from *Synechococcus spec.* (formerly *Anacystis nidulans*) absorb at 620 and 608 nm, respectively, indicating the α-subunit bearing the "f", the β-subunit bearing two "s"-type chromophores (Glazer et al. 1973). The superposition of the spectra of the two subunits does not yield that of the native protein, however. Such an additivity has been observed for a C–PE (Zickendraht et al. 1980). It is also indicated from the data of Binder et al. (1972) and unpublished data of the author for the renatured subunits of other C–PC's. In this case, the β-subunit bearing two chromophores absorbs at longer wavelength than the α-subunit bearing a single chromophore, which would correspond to the spectral shape of the native $\alpha\beta$-monomer. Irrespective of this conflicting interpretation is the assignment of the two compounds of the absorption spectrum of C–PC to different chromophores supported by photochemical hole-burning experiments (Friedrich et al. 1980a, b), by partial denaturation (Scheer and Kufer 1977) and chemical modification, e.g., with sodium dithionite (Kufer and Scheer 1979). In each case, differential effects on the two absorption bands are observed.

The bandwidth of the individual components is a narrow as in the purpurins (Fig. 5) (Scheer et al. 1977), or in the macrocyclic analogs, the chlorophylls. This is a good indication of a fixation of each of the chromophores, although possibly to different degrees. Similar results have been obtained for PE, although the analysis is more difficult due to the larger number of chromophores present. Reaction with sodium dithionite is preferential for the short-wavelength forms C–PE from *Pseudoanabaena* spec. W. 1173 (Kufer and Scheer 1979), and differential reactions have been observed too with organic mercury compounds (Pecci and Fujimori 1967, 1968). The subunits of this pigment have been analyzed in detail by fluorescence (Zickendraht-Wendelstadt et al.

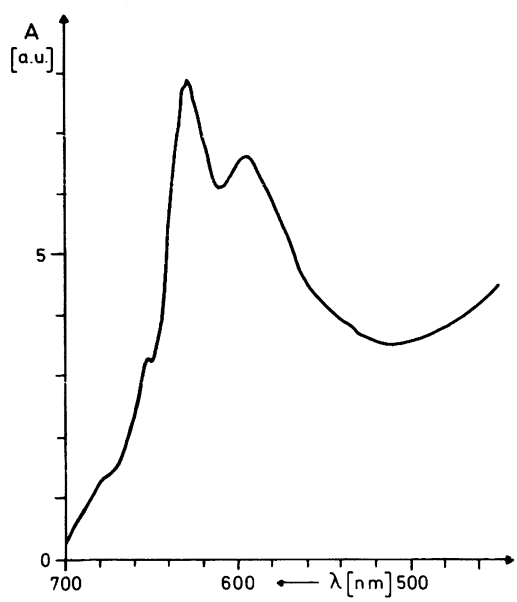


Fig. 5. Low-temperature spectrum of C–PC from *Spirulina platensis*, according to Scheer and Kufer (1977). The separation of the two bands corresponding to different chromophores is still increased at liquid helium temperatures. Only the long-wavelength peak is shown in Fig. 6

1980), and CD spectroscopy (Langer et al. 1980), and the results allow the distinction of all chromophores in the subunits, and of at least three of the five chromophores in the monomer.

The distinct environments of different chromophores in biliproteins is supported by analysis of the primary structure of chromopeptides and entire biliproteins. In all cases studied, there is a defined sequence to each chromophore, which is different for each chromophore in a given pigment, but similar for the corresponding chromophores in pigments from other organisms (Frank et al. 1978; Freidenreich et al. 1978; Otto et al. 1977; Troxler 1980; Zuber 1978).

A rigid fixation is necessary (although not sufficient, see below) to minimize radiationless decay of the excited states of biliproteins. This is accompanied by a narrowing of the absorption bands which is principally unfavorable to their antenna functions for two reasons: Only a narrow wavelength range is absorbed efficiently, and the overlap between emission bands of a sensitizing chromophore and the absorption of the next member of the Förster transfer chain becomes more crucial. Both effects are overcome by the development of chromophores absorbing at defined, closely spaced intervals, as realized most impressively in the PE's from red and cryptophytan algae, and in the phycobilisome superstructures (see Sect. 2).

Another necessary requirement for the suppression of radiationless processes is the suppression of photochemistry. This channel is important in the phytochromes and phycochromes (Björn 1979; Rüdiger 1980; Scheer 1981). Especially the results obtained recently in the latter case (see Sect. 6) indicate that small changes in the protein structure are already sufficient to open the photochemical channel, e.g., to increase radiationless transitions. Free bile pigments (Falk and Neufingerl 1979; Hudson and Smith 1975; Lightner 1977; Mac Donagh 1979; Manitto and Monti 1972), especially the A-Dihydrobilins (Kraus et al. 1979; Scheer and Krauss 1977; Scheer et al. 1977), are photolabile, but they react only with small quantum yields due to the competing radiationless deexcitation. It thus appears that the major effect of the protein is to decrease the latter processes, wile the change from efficient fluorescence to efficient photochemistry requires comparatively small modifications in the protein environment.

A subtle form of photochemistry, photoautomerization, is discussed as another pathway for rapid radiationless deexcitation of free bile pigments. Although at present no quantitative analysis has been done to assess its contribution in a certain pigment, examples are known from both bile pigments (Falk et al. 1979; Holzwarth et al. 1978) and other pigments (Schneider et al. 1979). Recently, photochemical hole-burning experiments have been performed on biliproteins (Friedrich et al. 1980a,b) which may ultimately help to answer this question, and give more insight into the protein-chromophore interaction. In this technic, a narrow, low-intensity laser beam is used to excite a glassy sample at low temperatures. Under certain conditions a narrow dip ("hole") in produced in the absorption band (Fig. 6) which may be accompanied by other holes, at longer wavelengths, and by a depression at the long-wavelength-side of the hole. The phenomenon is produced by a site-selective photochemical process, and the mechanism of this process has been studied in detail on small molecules. In hydroxyanthraquinones it could be identified as a hydrogen transfer process of a triplet state intermediate, which produces a thermolabile product absorbing at shorter wavelengths than the educt (Haarer et al. 1980), thus leaving a hole in the absorption envelope with its width deter-

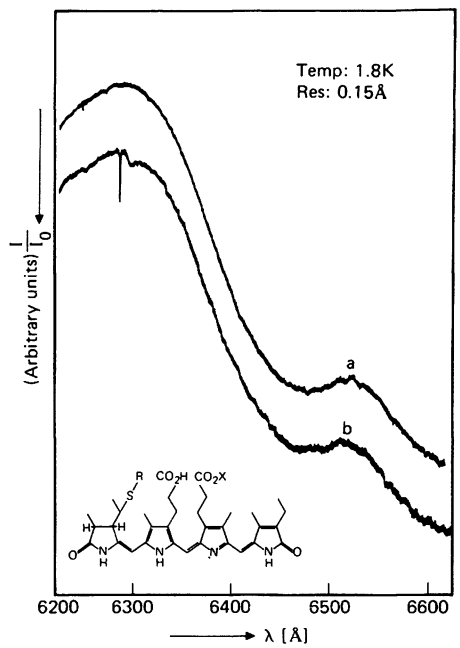


Fig. 6. Hole-burning experiment in C–PC from *Spirulina platensis*, according to Friedrich et al. (1980). Only the long-wavelength peak of the two visible bands of the low-temperature spectrum (Fig. 5) is shown in trace *a*. Trace *b* is obtained after 1 min irradiation with light (605 nm). The narrow hole has a half-width of 1.8 cm^{-1}, it has a small side band at longer wavelengths corresponding to a weak phonon coupling. Similar holes are obtained by irradiation of the APC band at 655 nm. In C–PE, additional narrow holes are produced, probably due to energy transfer between chromophores

mined by the relaxation kinetics. The situation is depicted in the term scheme (Fig. 7). Hole burning has been observed in APC, C–PC, C–PE, and in *8* as a model for the chromophores of the former two pigments, and the same mechanism (proton transfer from a triplet intermediate) has been suggested from the similar phenomenology. Bile pigment triplets have recently been studied by Land (1979).

Obviously, all three pigments can undergo low-temperature photochemistry. Processes of this type may account for the radiationless losses of isolated biliproteins, which are in the range of 20%–50% at ambient temperature (see Grabowski and Gantt 1978a) and do not change considerably at lower temperatures (Zickendraht-Wendelstadt et al. 1980). In the intact antenna, the energy transfer to Chl *a* is obviously much more rapid than photochemical processes, as transfer efficiencies of 99% have been determined experimentally. Further important information can be extracted from an analysis of the hole-burning features. In the case of the biliproteins, a small phonon coupling (corresponding to unfavorable Franck-Condon factors for radiationless decay) has been demonstrated, and the results for C–PE indicate a rapid energy transfer process among a highly ordered chromophore assembly. According to these results, the chromophores are well isolated from the protein matrix with regard to energy losses, but tightly coupled at the same time with regard to conformational restrictions.

An indirect form of protein-chromophore interactions is manifested in chromophore-chromophore interactions. There is increasing evidence for interactions of this type, mainly from CD spectra. S-shaped spectra or subspectra have been observed in APC's (Canaani and Gantt 1980), a cryptophytan PC (Jung et al. 1980), and a C-PE (Langer et al. 1980). A distinction between exciton coupling, or different chromophores with Cotton effects of opposite sign as the origin for these S-shaped bands is not trivial. The native *α*-subunit of C–PC from *Spirulina platensis* has a positive long-wavelength

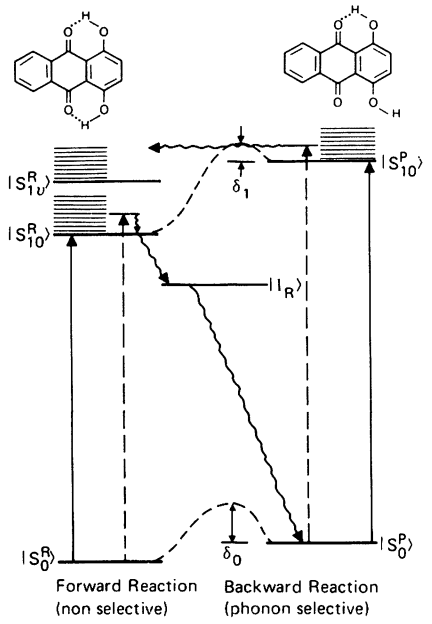


Fig. 7. Term scheme for 1,4-dihydroxyanthraquinone (*left*) and its photoproduct (*right*) according to Haarer et al. (1979). S_0 and S_1 are ground state and first excited singlet states, respectively. For the latter, the vibrational ground state S_{10} and a vibrational state ($S_1\gamma$) as well as additional phonon sublevels are shown, The superscripts refer to the educt (R) and the product (P). I_R is an intermediate, probably a triplet, the formation of which is rate-determining for the photoreaction. A similar term scheme is suggested for the biliproteins from hole-burning experiments like the one shown in Fig. 5

CD-band (Lehner and Scheer 1981), whereas P_T bearing a structurally closely related chromophore of the same absolute configuration exhibits a negative effect (Brandlmeier et al. 1981). Since the CD-spectra of both pigments bearing only a single chromophore, are very similar in the denatured state, the opposite sign is due to chromophore-protein interactions of opposite chirality. It is then difficult to distinguish in pigments bearing up to six chromophores, between chromophore-protein interactions of opposite chirality on different chromophores, and S-shaped bands originating from exciton coupling. The former is supported from the correlation of the absorption maxima with the points of zero ellipticity in the CD spectra of APC, and controled denaturation experiments (similar decrease of the positive and negative extrema in C–PE).

Intermediate strength (viz. CD-incative) couplings between chromophores have been discussed for chromophores on different proteins in the APC-trimer (Mac Coll et al. 1980). The absorption of native APC is shifted from 620 to 650 nm and increased in intensity upon trimerization (Brown et al. 1975; Cohen-Bazire et al. 1977; Erokhina and Krasnovskii 1974; MacColl et al. 1981), similarly striking CD-changes, and less pronounced absorption changes have been observed for other biliproteins as well upon dissociation (Bennett and Bogorad 1971; Glazer et al. 1973; Gray and Gantt 1975). The CD changes observed recently upon controled dissociation of phycobilisomes have been interpreted on the same basis (Rigbi et al. 1980).

6 The Proteins

Protein structure and aggregation of biliproteins have been summarized recently in several reviews and shall be discussed only briefly in this context. One of the aspects studied in more detail is the phylogenetic relationship between the various biliproteins.

The family tree shown in Fig. 8 has been derived from sequenation studies and immunochemical investigations (Zuber 1978). The completed sequences of two C–PC's (Frank et al. 1978; Troxler 1980), an APC (Zuber et al. 1981) and of one subunit each of another C–PC (Freidenreich et al. 1978), principally support this picture, but there is evidence for very pronounced variations with sequenation studies of a marine cayno-bacterium *Agmenellum quadruplicatum* (Fox 1980). The completed sequences also indicate the presence of conservative as well as of more variable regions, the former being associated with the chromophore environments (see Muckle et al. 1977; Zuber 1980). This part may well be crucial for the efficiency and stability of the chromophores.

Due to conflicting earlier results (Berns 1967; Glazer et al. 1971), the cryptophytan biliproteins had not been included in Fig. 8. Recent immunological evidence supports a relationship of both cryptophytan PC and PE with rhodophytan PE, but neither with rhodophytan PC nor cyanobacterial pigments (MacColl et al. 1976). This is yet another aspect of the somewhat special character of these pigments, since in both cyanobacterial and rhodophytan pigments the immunology parallels the spectroscopic classification. But it also points to the increasing complexity of biliproteins emerging with an increasing number of pigments studied. Phycoerythrocyanin and R–PC are other examples of pigments with mixed chromophores, which could be correlated to the "common" pigments only by immunochemical methods (see Table 2 for references). Another example is the increasing number of biliproteins containing a γ-chain, which may have to be added to the family tree as another branch. Besides R– and B–PE, which form an $\alpha_6\beta_6\gamma$-structure, (Abad-Zapatero et al. 1977; Gantt and Lipschultz 1974; Sweet et al. 1977), APC–I with probably $\alpha_3\beta_3\gamma$-structure is the third example of this class (Zilinskas et al. 1978).

One of the most prominent properties of cyanobacterial and rhodophytan biliproteins is their pronounced aggregation. Especially the aggregation of PC's has been studied in great detail. Monomers, trimers, and hexamers are the most abundant species in solutions of the purified pigments (MacColl et al. 1971), but dimers (Iso et al. 1977) and tetramers (Neufeld and Riggs 1969) have been observed, too. The influence of various parameters including concentration (Lee and Berns 1968; MacColl et al. 1974), ionic strength (MacColl et al. 1971a), pH (Lee and Berns 1968; Saito 1976), temperature (MacColl et al. 1971a), aromatic compounds (MacColl and Berns 1973), substitution of [^{1}H] by [^{2}H] (Hattori et al. 1965; Lee and Berns 1968), inorganic and organic salts (Berns and Morgenstern 1968; MacColl et al. 1971b), biotope of the parent organisms (Chen and Berns 1979; Kao et al. 1973, 1975), and others (Chen and Berns 1978), have been investigated. A major contribution of hydrophobic interactions as the driving force for aggregation has been concluded.

The trimers and hexamers are the building blocs both of phycobilisomes (Gantt 1975; Glazer et al. 1979; Mörschel et al. 1980) and biliprotein crystals (Abad-Zapatero et al. 1977; Bryant et al. 1976; Dobler et al. 1972; Sweet et al. 1977). Accordingly, higher aggregates (Berns 1971; MacColl and Berns 1979) as well as hetero-aggregates (Grabowski et al. 1980; Koller et al. 1978; Rigbi et al. 1980) can be obtained from controled dissociation of the former. In view of the architecture of the phycobilisomes, the γ-chain in R– and B–PE as well as in APC–I may then serve as a further means to stabilize the trimeric and hexameric building bloc, forming a core around which the

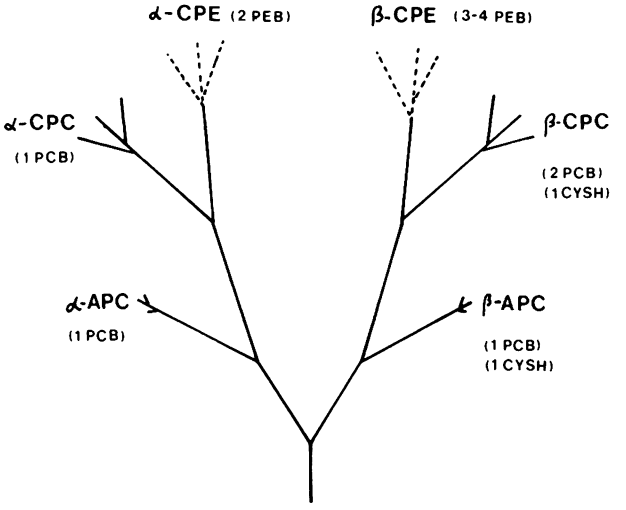


Fig. 8. Family tree of phycobiliproteins, according to Zuber (1978), as derived from sequenation studies and immunochemical relationsships. The abbreviations refer to the chromophores present (*PCB = 1, PEB = 2*) or to free cystein residues (*CYSH*)

α- and β-subunits are arranged. It is interesting in this respect, that PC-hexamers isolated from phycobilisomes do not show the expected "inner hole" in negatively stained electron micrographs, and may contain a colorless core-protein instead (Mörschel et al. 1980). The cryptophytan biliproteins do not form similar large and stable aggregates, corresponding to the absence of phycobilisomes in these organisms (see Gantt 1979).

7 Biosynthesis

The name biliprotein is derived from these pigments bearing chromophores structurally similar to the bile pigments of higher animals. The latter are derived from oxidative breakdown of the porphyrin macrocycle of hemes, which is apparently the only physiological way to preserve the iron for recycling (MacDonagh 1978; O'Carra 1975). From this point of view, the bile pigments are then only waste products of this process. By contrast, bile pigments in cyanobacteria, red and cryptophytan algae, are synthesized for the essential function of photosynthesis.

In spite of this teleological difference, the biosynthesis of the two types of bile pigments is surprisingly similar in several aspects. Both are derived from protoporphyrin (IX), in both cases the macrocycle is opened at the C–5 position (see Troxler 1976). Apparently, the mechanism of the ring opening is similar, too. One molecule of CO is released (Troxler 1972), and two molecules of oxygen yield each one oxygen atom to the bile pigments (Troxler et al. 1979). It is not yet clear, whether the educt for this ring opening is an iron porphyrin in the chromophore biosynthesis of biliproteins. Chemical model studies support a Mg-porphyrin as a precursor as well (Barrett 1967; Hudson and Smith 1975), and a tetrahydroporphyrin with an ethylidene group at the

proper position is known with bacteriochlorophyll *b* (Scheer et al. 1974). Brown et al. (1980) have, however, recently demonstrated the incorporation of exogenous hemin into phycobilins, but not chlorophylls in *Cyanidium caldarium*, which strongly supports the "iron-pathway" in the former.

Nothing is yet known as to the point at which during biosynthesis the chromophore is linked to the protein. Facets to this problem are (1) the finding of various strains of *Cyanidium caldarium* which excrete after treatment with δ-aminoevulinic acid (ALA), the ethylidene bilin *4* into the medium, together with products derived from addition reactions at the ethylidene function (Troxler and Bogorad 1966; Troxler et al. 1978); (2) the hitherto unsucessful search for the free apoprotein of any of these pigments; and (3) the demonstration of reversible addition reactions to the 3-ethylidene bond of *4* (Beuhler et al. 1976; Gossauer et al. 1980; Klein and Rüdiger 1978). Taken together, they indicate that the bilin *4* may be the biosynthetic precursor, which is attached to the protein in one of the last steps.

The biosynthesis of biliproteins is effected by several nutritional factors, of which nitrogen (see Bogorad 1975), sulfur (Schmidt 1980) and especially light, are studied in detail. Light regulates the synthesis of both chlorophylls and biliproteins. The tetrapyrole skeletons of both pigments are derived from ALA and share a common part of their biosynthetic pathways, although possibly not their ALA pool. In a recent study, with mutants of *Cyanidium caldarium* defective in chlorophyll or biliprotein biosynthesis, Schneider and Bogorad (1979) obtained evidence for two different, but strongly interacting photoregulation systems. A protochlorophyll-type and a hemoprotein-type pigment, respectively, may be involved as photoreceptors. For leading references on light-dependent development in cyanobacteria and red algae the reader is referred to: Björn 1979, 1980; Bogorad 1975; Bogorad et al. 1975; Lazaroff 1973.

In many cyanobacteria and red algae, light-stimulated developmental responses have been observed, which suggest the presence of photoreversibly photochromic pigments as photoreceptors (see Björn 1979; Björn 1980; Bogorad 1975) (Table 4a). Chromatic adaptation is a widespread response, by which (among other effects) the composition of the biliprotein antenna is changed with the environmental light quality. The relative amount of green-light absorbing PE's is reversibly increased in green light and decreased in red light (Bogorad 1975; Lazaroff et al. 1973; Tandeau de Marsac 1977; Wagenmann 1977), with a concomitant change in phycobilisome composition (Siegelman 1980). Another light-mediated response is the induction of filamentous growth, e.g., in *Nostoc* (Ginsburg and Lazaroff 1973). Corresponding to these effects, the terms adaptochromes and phycomorphochromes have been suggested (Bogorad 1975). In analogy to phytochrome, the photoreversible photochromic sensory pigment of higher plants (Rüdiger 1980), the term phycochromes is often used synonymously for these receptors.

It my be useful to reserve the term phycochromes to photoreversibly photochromic pigments irrespective of any proven function as a photoreceptor for the present time (see Björn 1979, for a discussion). This distinction arose from the attempts to isolate adaptochromes and phycomorphochromes, e.g., the genuine photoreceptors. During this search, Scheibe (1972) first isolated a biliprotein fraction from photobleached *Tolypothrix tenuis*, which gave photoreversible absorption differences reminiscent of the action spectra for chromatic adaption in this species. The Björns have

since characterized four different phycochromes (*a, b, c, d*) from various organisms, and purified to a different degree (Björn 1978; Björn and Björn 1976). All appear to be biliproteins, and thus require very tedious and extensive purification from the bulk pigments, with isoelectric focusing as the key step. In only one of the photochromic fractions, the absorption differences exceed a few percent, although the quantum yields are reasonable. Surprisingly, only two of these four phycochromes (Table 4b) can presently be correlated spectroscopically to a known light response in the parent organisms, which led to the aforementioned distinction of phyochromes (pigments with photoreversible photochromic properties) on one hand, and adaptochromes and phycomorphochromes (as photoceptors for the respective processes) on the other. To link these chemical and functional properties to a certain pigment, it may be necessary to obtain further information besides the spectral data. As one such possibility, a differential temperature effect on the forward and back reaction of the photoreversion process has been suggested recently (Ohad et al. 1979).

The need for a distinction becomes even more obvious from recent results on the photochemical properties of phycobiliproteins partially denatured with low concentrations of guanidinium chloride (Ohki and Fujita 1979) or a decrease in pH (Ohad et al. 1979, 1980) (Table 4c). By this treatment, APC and PC obtain photoreversible photochromic properties reminiscent of the phycochromes *a* and *c* respectively, of Björn and Björn (1976). According to all evidence, this is no artifact due to a co-isolated impurity. It rather appears that at least in these cases phycochromes are not separate pigments, but rather slightly denatured states of well-known biliprotein antenna pigments, or certain sub-populations there of. It is interesting in this respect that phycochrome *d* has been associated recently with another biliprotein, phycoerythrocyanin (Björn 1980). The denaturant-induced photochromicity of PC and APC decreases again at more severe denaturation conditions. Probably, the "tickling" of the protein looses the interactions with the protein sufficiently to open a photochemical channel, while internal conversion and destructive photochemistry of the pigments are still inhibited. More severe uncoupling (see Sect. 5) then favors the latter processes. Stepwise denaturation has been observed with PC *Spirulina platensis*, suggesting a distinct intermediate in the unfolding process (Scheer and Kufer 1977). Similar intermediates may be produced in the pigments isolated from *T. tenuis* (Ohki and Fujita 1979) and *F. diplosiphon* (Ohad et al. 1979, 1980). MacColl et al. (1980b) recently pointed out, that similar difference spectra as in the phycochrome reaction, are observed in the reversible dissociation of trimeric $(\alpha\beta)_3$ *APC II*. It would then be crucial to link the dissociation-reassociation unequivocally to the light reaction rather than to an artefact, e.g., due to sample heating.

8 Concluding Remarks

This review on phycobiliproteins is far from complete. This is not only due to the widespread and still growing interest in these pigments, which is prohibitive to the citing of all pertinent work, but also to the deliberate attempt of the author, to focus mainly on the chromophore protein interactions. These interactions in the native biliproteins are crucial for their functions, and only recently some general principles have been recog-

Table 4. Action maxima for light-stimulated developmental responses in whole organisms (a), for reversible absorption changes in biliprotein fractions (b), and for reversible absorption changes in partially denatured, purified biliproteins

	"green" form		"red" form		References
	λ_{max}	action	λ_{max}	action	
(a) Action spectra for Photoresponse (chromatic adaptation or photomorphose) in whole organisms					
Tolypothrix tenuis	541 550, 350	PE formation	641 660, 360	PC formation	Fujita and Hattori (1962) Diakoff and Scheibe (1973) Ohki and Fujita (1978)
Fremyella diplosiphon	550, 387, 540	PE formation	641, 463, 650, 360	PC formation	Vogelmann and Scheibe (1978) Haury and Bogorad (1977)
Nostoc muscorum	~550	Reversion	650	Induction of filamentous growth	Lazaroff and Schiff (1962)
Nostoc commune	520		640		Robinson and Miller (1970)
Cyanidium caldarium [c]	420, 550, 595	PBP formation	–	–	Nichols and Bogorad (1962) Schneider and Bogorad (1979)
(b) "Action spectra" for reversible absorption changes in algal extracts or enriched fractions of phycobiliproteins ("phycochromes")					
"Photoreversible Pigment" from photobleached *Tolypothrix tenuis*	~520	formation P_{650}	650	reversion	Scheibe (1972) Ohki and Fujita (1979a)
Phycochrome *a* from: *Phormidium luridum, Anabaenae, Tolypothrix distorta*	630	formation P_{580}	580	reversion	Björn and Björn (1978) Björn (1980)

Phycochrome *b* from *Tolypothrix distorta, Anabaenae* [b]	580	formation P_{500}	500	reversion	Björn and Björn (1976) Björn (1980)
Phycochrome *c* from *Nostoc muscorum*	630, 650[a]				Björn and Björn (1976) Björn (1980)
Phycochrome *d* from *Tolypothrix distorta Anabaenae*[b]	650	formation P_{650}	610 – 620	reversion	Björn (1978, 1980)
APC in phycobiliprotein mixtures obtained by isoelectric focusing (pI = 4.4.3) of extracts from *Fremvella diplosiphon* and *Nostoc muscorum*	645	formation P_{555}	560	reversion	Ohad et al. (1980)
(c) "Action spectra" for reversible absorption changes in partially denatured, purified biliproteins					
PC from *Tolypothrix tenuis* "tickled" with 0.5 M guanidinium	570	formation P_{630}	630	reversion	Ohki and Fujita (1979b)
APC 0.5 M guanidinium chloride	600	formation P_{650}	650	reversion	
APC from *Fremyella diplosiphon, Nostoc muscorum* and *Phormidium luridum* at pH 4	645	formation P_{555}	550 – 560	reversion	Ohad et al. (1980)

a Probably two different forms

b A correlation has recently been indicated between the occurence of phycochromes *b* and *d* and the presence of phycoerythrocyanin in the respective organism (G.S. Björn, to be published)

c Probably no photoreversibly photochromic pigment, although with an action spectrum similar to such pigments from *F. diplosiphon*

nized. Most details are derived from spectroscopic and chemical studies of the chromophores. The protein environment is known only for a few pigments in its primary structure, and the amino acid residues responsible for the noncovalent interaction in the native pigments are still unknown. X-ray structure, as the most promising tool to study these interactions, has encountered unexpected difficulties, but may provide these data in the near future. Other major gaps in our present knowledge concern the last steps in the biosynthesis of biliproteins, the processes of biliprotein aggregation to phycobilisomes, and the details about the phycobilisome-membrane junction critical for energy transfer to the reaction centers. It should also be borne in mind that biliproteins have been studied in detail only from a small number of species.

Phycobiliproteins are a good example of molecular ecology, and they may help not only in understanding better the processes of photosynthesis, but also the interactions between proteins and small molecules in general. This, together with the fun to work with these brilliantly colored pigments, is expected to fill at least part of the gaps in the near future.

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Notes Added in Proof

This manuscript contains references acessible to the author until mid 1981. Some recent progress is added below. The reader is also referred to a review on phycobilisomes and phycobiliproteins by R. MacColl to appear in Photochem. Photobiol.

Energy Transfer

The transfer efficiencies from R–PE to R–PC (93%), R–PC to "bulk" APC (98%) and "bulk" APC to the "terminal" APC (96%) have been determined for a rhodophytan phycobilisome [Bekasova et al.; Biofizika **26**, 74 (1981)]. Several groups are investigating the energy transfer in biliproteins and phycobilisomes by picosecond time-resolved techniques. By comparison of low and high intensity excitation, $^1S-^1S$ annihilation has been suggested as a complication both in isolated biliproteins [Wong et al.; Photochem. Photobiol. **33**, 651 (1981); Doukas et al. ibid. **34**, 505 (1981)] and in phycobilisomes (Holzwarth et al., unpubl.). Attempts to analyze the complex fluorescence or ground-state recovery are ambiguous, since good fits can be obtained using expotentials only, but also including nonlinear exponents ($t^{-1/2}$) originating from a Förster mechanistic analysis (Holzwarth et al., unpublished; Hefferele, Nies, Wehrmeyer and Schneider, unpublished). Depolarization studies have been included in the latter study and also in denaturation work with C–PC in defined aggregation states (Hefferle, John, Scheer and Schneider, unpublished) to aid this analysis. Brody et al. (Biophys. J. **34**, 439 (1981) reported the puzzling result, that the 715 nm fluorescence (generally associated with chlorophyll) arose faster than that of biliproteins when exciting the latter, which may indicate alternative transfer schemes, e.g., via carotenoids (Szalontay and v.d. Ven, FEBS Lett. **131**, 155 (1981).

Structure of Phycobilisomes and Reconstitution of Functional Complexes

Kirilowski and Ohad (Proc. Natl. Acad. Sci. USA, in press) reported the reassembly of fully dissociated phycobilisome-membrane complexes, and Glick and Zilinskas (Plant Physiol., in press) demonstrated the importance of the structural peptides by reassembling phycobilisomes from the individual pigments in the presence of a colorless 29 kdalton peptide. The function and properties of the large structural peptide (75-90 kdalton) have been studied in some detail and the results seem to rationalize earlier conflicting results. This colored peptide is readily susceptible to bleaching and proteolysis [Lundell and Glazer; J. Biol. Chem. **256**, 12600 (1981); Lundell et al., J. Cell. Biol. **91**, 315 (1981); Ruschkowski, Ph. D. Thesis, Rutgers University, 1981] and is probably the genuine γ-subunit of APC–I, serving as the terminal energy donor to chlorophyll in these algae, and as (one of) the anchor-protein(s) of the phycobilisome to the membrane. The facile proteolysis of this peptide is reminiscent of other peripheral membrane proteins, e.g., some b-cytochromes. The functionality and structure of phycobilisomes during N-starvation has been studied by Yamanaka and Glazer [Arch. Microbiol. **124**, 39 (1980)]. The APC core and adjacent PC hexamers essential for energy transfer are kept functional until a very late stage of starvation, whereas the phycobilisome periphery including specific structural peptides are digested. Nies and Wehrmeyer [Arch. Microbiol. **129**, 374 (1981)] studied in some detail the phycobilisomes from *M. laminosus* and its dissociation products to arrive at a model very similar to that of the red algae, *R. violacea* shown in Fig. 1.

Isolated Biliproteins

The primary structure of a third C–PC (*C. caldarium*) solved by Troxler et al. [J. Biol. Chem. **256**, 12176 (1981)] and Offner et al. [ibid. p. 12167] supports the homology between pigments from different species. Murakami et al. [J. Biochem. **89**, 79 (1981)] studied the reversible aggregation of APC under varying environmental conditions. By curve-resolution of the absorption band, they concluded that one of the two chromophores is unaffected by this process, whereas the second chromophore is blue-shifted and decreased in intensity in the monomer [Mimuro et al., Arch. Biochem. Biophys. (in press)]. This change is similar to the change observed upon deprotonation of bile-pigment cations to the free bases (see text). Yu et al. [Plant. Physiol. **68**, 482 (1981)] found a variation in the chromophore composition indicating an exchange of phycourobilin with phycoerythrobilin chromophores in R–PE of two closely related species, and also in a single species by chromatic adaptation.

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