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Editors

A. Pirson, Göttingen
M.H. Zimmermann, Harvard

Photo-
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Edited by
W. Shropshire, Jr. and H. Mohr

Contributors

K. Apel M. Black A.E. Canham J.A. De Greef M.J. Dring
H. Egnéus B. Frankland H. Frédéricq L. Fukshansky
M. Furuya V. Gaba A.W. Galston J. Gressel W. Haupt
S.B. Hendricks M.G. Holmes M. Jabben H. Kasemir
C.J. Lamb M.A. Lawton K. Lüning A.L. Mancinelli
H. Mohr D.C. Morgan L.H. Pratt P.H. Quail R.H. Racusen
W. Rau W. Rüdiger E. Schäfer H. Scheer J.A. Schiff
P. Schopfer S. D. Schwartzbach W. Shropshire, Jr.
H. Smith W.O. Smith R. Taylorson W.J. Van DerWoude
D. Vince-Prue H.I. Virgin E. Wellmann

With 173 Figures

*13 42440 *8*

W. Shropshire, Jr.
Smithsonian Institution
Radiation Biology Laboratory
12441 Parklawn Drive
Rockville, MD 20852/USA

H. Mohr
Biologisches Institut II der Universität
Lehrstuhl für Botanik
Schänzlestr. 1
D-7800 Freiburg/FRG

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List of Contributors Part A and B

K. APEL
  Botanisches Institut
  der Universität Kiel
  Olshausenstraße 40–60
  D-2300 Kiel/FRG

M. BLACK
  Department of Biology
  Queen Elizabeth College
  (University of London)
  Campden Hill Road
  London W8 7AH/United Kingdom

A.E. CANHAM
  University of Reading
  Department of Agriculture and
  Horticulture
  Earley Gate
  Reading, Berks. RG6 2AT/
  United Kingdom

J.A. DE GREEF
  Department of Biology
  University of Antwerpen
  (UIA-RUCA)
  Universiteitsplein 1
  B-2610 Wilrijk/Belgium

M.J. DRING
  Botany Department
  Queen's University
  Belfast BT7 1NN/United Kingdom

H. EGNÉUS
  University of Göteborg
  Botanical Institute
  Department of Plant Physiology
  Carl Skottsbergs Gata 22
  S-413 19 Göteborg/Sweden

B. FRANKLAND
  School of Biological Sciences
  Queen Mary College
  (University of London)
  Mile End Road
  London EI 4NS/United Kingdom

H. FRÉDÉRICQ
  Laboratory of Plant Physiology
  University of Gent
  Ledeganckstraat, 35
  B-9000 Gent/Belgium

L. FUKSHANSKY
  Institut für Biologie II/Botanik
  Schänzlestraße 1
  D-7800 Freiburg/FRG

M. FURUYA
  Department of Biology
  Faculty of Science
  University of Tokyo
  Hongo, Tokyo, 113/
  Japan

V. GABA
  Department of Biology
  Queen Elizabeth College
  (University of London)
  Campden Hill Road
  London W8 7AH/United Kingdom

A.W. GALSTON
  Department of Biology
  Yale University
  P.O. Box 6666
  New Haven, Connecticut 06511/
  USA

J. GRESSEL
  The Weizman Institute of Science
  Department of Plant Genetics
  Rehovot, 76100/Israel

W. HAUPT
  Institut für Botanik
  und Pharmazeutische Biologie
  der Universität Erlangen-Nürnberg
  Schloßgarten 4
  D-8520 Erlangen/FRG

S.B. HENDRICKS
  (Deceased)

M.G. HOLMES
Smithsonian Institution
Radiation Biology Laboratory
12441 Parklawn Drive
Rockville, Maryland 20852/USA

M. JABBEN
Max-Planck-Institut
für Strahlenchemie
Stiftstraße 34–36
D-4330 Mühlheim/FRG

H. KASEMIR
Institut für Biologie II/Botanik
Schänzlestraße 1
D-7800 Freiburg/FRG

C.J. LAMB
The Salk Institute for Biological
Studies
P.O. Box 85800
San Diego, California 92136/USA
and
Plant Biology Laboratory
10010 North Torrey Pines Road
La Jolla
San Diego, California 92138/USA

M.A. LAWTON
Department of Biology
Washington University
Campus Box 1137
St. Louis, Missouri 63130/USA

K. LÜNING
Biologische Anstalt Helgoland
Notkestraße 31
D-2000 Hamburg 52/FRG

A.L. MANCINELLI
Department of Biological Sciences
1108 Schermerhorn Hall
Columbia University
New York, NY 10027/USA

H. MOHR
Biologisches Institut II
der Universität
Lehrstuhl für Botanik
Schänzlestraße 1
D-7800 Freiburg/FRG

D.C. MORGAN
D.S.I.R.
Plant Physiology Division
Private Bag
Palmerston North/New Zealand

L.H. PRATT
Botany Department
University of Georgia
Athens, Georgia 30602/USA

P.H. QUAIL
Department of Botany
139 Birge Hall
University of Wisconsin-Madison
Madison, Wisconsin 53706/USA

R.H. RACUSEN
Department of Botany
University of Maryland
College Park, Maryland 20742/USA

W. RAU
Botanisches Institut der Universität
Menzinger Straße 67
D-8000 München 19/FRG

W. RÜDIGER
Botanisches Institut der Universität
Menzinger Straße 67
D-8000 München 19/FRG

E. SCHÄFER
Institut für Biologie II/Botanik
Schänzlestraße 1
D-7800 Freiburg/FRG

H. SCHEER
Botanisches Institut der Universität
Menzinger Straße 67
D-8000 München 19/FRG

J.A. SCHIFF
Brandeis University
Institute for Photobiology
of Cells and Organelles
South Street
Waltham, Massachusetts 02154/USA

P. SCHOPFER
Biologisches Institut II
der Universität
Lehrstuhl für Botanik
Schänzlestraße 1
D-7800 Freiburg/FRG

S.D. SCHWARTZBACH
Genetics, Cellular and Molecular
Biology Section
School of Life Sciences
348 Manter Hall
University of Nebraska-Lincoln
Lincoln, Nebraska 68588-0118/USA

W. SHROPSHIRE, JR.
   Smithsonian Institution
   Radiation Biology Laboratory
   12441 Parklawn Drive
   Rockville, Maryland 20852/USA

H. SMITH
   Department of Botany
   University of Leicester
   University Road
   Leicester LE1 7RH/
   United Kingdom

W.O. SMITH
   Smithsonian Institution
   Radiation Biology Laboratory
   12441 Parklawn Drive
   Rockville, Maryland 20852/USA

R. TAYLORSON
   U.S. Department of Agriculture
   Bldg. 001 Rm. 40 BARC-West
   Beltsville, Maryland 20705/USA

W.J.VANDERWOUDE
   Light and Plant Growth Laboratory
   Beltsville Agricultural
   Research Center
   Beltsville, Maryland 20705/USA

D. VINCE-PRUE
   Glasshouse Crops Research
   Institute
   Worthing Road
   Littlehampton
   West Sussex, BN17 3PU/
   United Kingdom

H.I. VIRGIN
   University of Göteborg
   Botanical Institute
   Department of Plant Physiology
   Carl Skottsbergs Gata 22
   S-413 19 Göteborg/Sweden

E. WELLMANN
   Biologisches Institut II
   der Universität
   Schänzlestraße 1
   D-7800 Freiburg/FRG

7 Chromophores in Photomorphogenesis

W. Rüdiger and H. Scheer

1 Introduction

Chromophores in photomorphogenesis are those parts of the photoreceptor molecules which absorb the light responsible for the physiological response. Absorption spectra of the chromophores should therefore principally correspond to the action spectra of photomorphoses. However, the absorption of isolated chromophores can strongly deviate from physiological action spectra due to several reasons (e.g., perturbation by the environment, dichroitic effects of ordered structures, shading by bulk pigments). Therefore, we restrict our discussion here to those chromophores on which at least some complementary information is available.

The chromophore of phytochrome has previously been treated in several books and reviews (MITRAKOS and SHROPSHIRE 1972, SMITH 1975, BRIGGS and RICE 1972, SMITH and KENDRICK 1976, KENDRICK and SPRUIT 1977, PRATT 1978, RÜDIGER 1980). A comprehensive bibliography on the literature prior to 1975 is available (CORRELL et al. 1977). Phycochrome and adaptochrome chromophores have been discussed by BOGORAD (1975) and BJÖRN and BJÖRN (1980). For a recent survey on cryptochrome (the blue light receptor) the reader is referred to the book edited by SENGER (1980).

2 Phytochrome Chromophores

2.1 P_r Structure

Because of spectral similarity of P_r and PC^1, a bile pigment structure was suggested for the phytochrome chromophore at an early stage of phytochrome research (PARKER et al. 1950). Subsequently, the biliproteins PC, APC and PE, and their chromophores phycocyanobilin and phycoerythrobilin which are readily available, have been used extensively as model compounds for phytochrome and its chromophores.

2.1.1 Degradation Studies

Chromic acid degradation of bile pigments and biliproteins under carefully controlled conditions leads to well-defined oxidation products, namely maleimids

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[1] Abbreviations: PC = phycocyanin, PE = phycoerythrin, APC = allophycocyanin
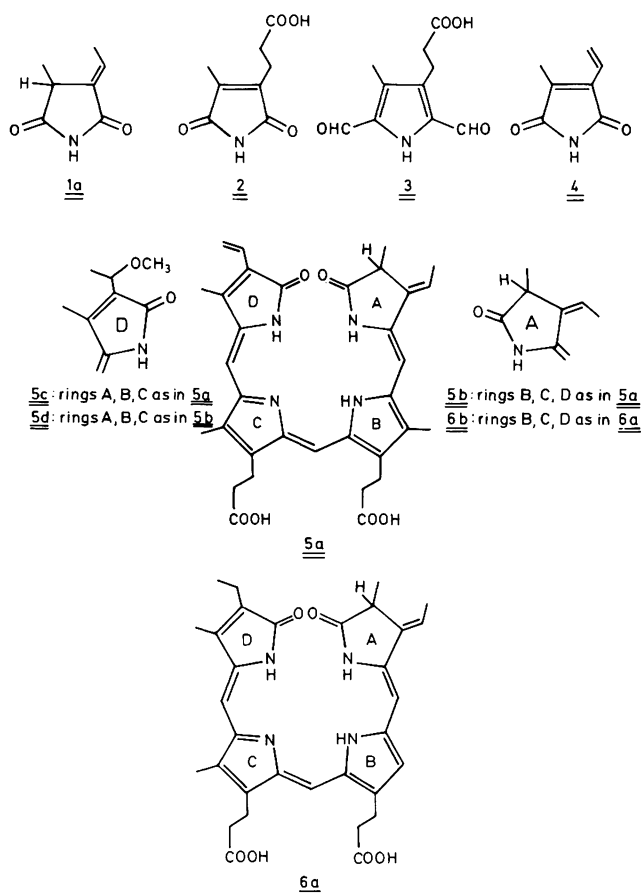
Fig. 1. Structure of phytochromobilin, related tetrapyrroles and degradation products thereof

and succinimides with typical substitution patterns. These products can be identified by thin layer chromatography and specific staining (RÜDIGER 1969, 1970). Porphyrins and chlorophylls yield the same or similar oxidation products, but bile pigments can be distinguished from these tetrapyrrols by oxidation at pH 0–1. Under these conditions, only bile pigments (and biliproteins) are degraded but no other tetrapyrrols.

Investigation of phytochrome with this method proceeded in several steps. With the first (denatured) sample, the bile pigment nature of the P_r chromophore was unequivocally confirmed (RÜDIGER and CORRELL 1969). Furthermore, the true degradation products from pyrrole rings B and C [(2) and (3); see Fig. 1] were obtained, whereas other products probably derived from rings A and D later turned out to be artifactual. The true degradation product from ring D (4) was only obtained 3 years later (RÜDIGER 1972). The key product from ring A (1 a) was only obtained by modified degradation procedure (chromic acid — ammonia degradation, KLEIN et al. 1977, KLEIN and RÜDIGER 1978)

which also cleaved the covalent linkage between ring A and the protein (see Sect. 2.1.5). In summary, the hypothetical structure 5a for free phytochromobilin was derived from these studies. Additional evidence for the protein binding was also derived from these studies (see Sect. 2.1.5). It should be kept in mind that degradation studies only allow the deduction of chromophore side chains. Structure (5a) differs from that of phycocyanobilin (6a) only by a formal exchange of an ethyl group for the vinyl group at ring D. The side chains of (5a) are identical with those of phycoerythrobilin, but the conjugated system is interrupted between rings C and D in the latter whereas the conjugation comprises all four rings in (5a) according to spectral studies.

2.1.2 Spectral Studies

Electronic spectra of free bile pigments consist of one broad band in the visible and possibly a second band in the near UV range. Mainly the visible band has been used extensively for classification and characterization of bile pigments (RÜDIGER 1971). Not only the position of this band, but also the shift induced

Table 1. Visible absorption maxima (nm) of some bile pigments and biliprotein chromophores related to phytochrome P_r

	Cation	Base	Zinc complex	References
Biliverdin (19a)[a]	700	653	715	RÜDIGER et al. (1968)
Mesobiliverdin[a]	685	630–655	688	KÖST et al. (1975)
Octaethylbiliverdin (26)[a]	693	657	691	SCHEER (1976)
Phytochromobilin (5a)[a]	690	–	–	SIEGELMAN et al. (1966)
	708	610	–	WELLER and GOSSAUER (1980)
Phycocyanobilin (6a)	687	603	628, 673	KÖST et al. (1975)
Phytochrome P_r (15)[b]	675–689	620–625	650 (590)	GROMBEIN et al. (1975)
Phycocyanin PC (28)[b]	665–670	610 (590)	640 (590)	GROMBEIN et al. (1975)
A-dihydrobiliverdin (20)[a]	665	594	638	SCHEER (1976)
A-dihydrobiliverdin (20)[c]	–	617 + 566	–	
Phycocyanobilin (6a)[c]	–	641 + 587	–	RÜDIGER et al. (1980)
Methanoladduct (5c)[c]	–	636 + 582	–	
Phytochromobilin (5a)[c]	–	653 + 600	–	

[a] Methanol
[b] 6 m guanidinium chloride
[c] Ethyl acetate

by derivatization (e.g., cation or zinc complex formation) is characteristic for the chromophore type. The data of Table 1 show that phytochromobilin fits into the series of fully conjugated bilins (formerly called bilatrienes).

Biliproteins cannot directly be compared with free bile pigments in this respect because, in the native state, spectral properties of bilin chromophores are drastically modified by the protein (see Sect. 2.1.6). But after unfolding of the peptide chain, biliproteins behave similarly to free bilins (KÖST et al. 1975, GROMBEIN et al. 1975). Phytochrome (P_r) and PC unfolded with guanidinium chloride are included in Table 1.

The data of Table 1 are consistent with structure (5a) for phytochromobilin. A small red shift compared with the data of phycocyanobilin (6a) can be explained by the increment of the vinyl group at ring D (see Fig. 2). This increment (vinyl versus ethyl) can also be observed in other bile pigments. Differing λ_{max} values reported for the cation of (5a) (SIEGELMAN et al. 1966, WELLER and GOSSAUER 1980) are probably due to slightly different conditions of measurement which could lead to different populations of bilin conformers in solution. This is a basic problem in bile pigment spectroscopy because it was shown that solutions of bile pigments mostly consist of mixtures of conformers with different spectral properties (BRASLAVSKY et al. 1980a, LEHNER et al. 1978a, 1979, HOLZWARTH et al. 1978, 1980, SCHEER et al. 1977, PÉTRIER et al. 1979; see also Sect. 2.1.6). These discrepancies are especially pronounced with the free bases (Table 1). Solutions of free bases contain sometimes two peaks in varying intensity or one peak with pronounced shoulders which can best be resolved by derivative spectroscopy (RÜDIGER et al. 1980).

biliverdin 19a

mesobiliverdin
octaethylbiliverdin 26

phytochromobilin 5a

phycocyanobilin 6a

phytochrome 15

phycocyanin
A–dihydrooctaethylbiliverdin 20
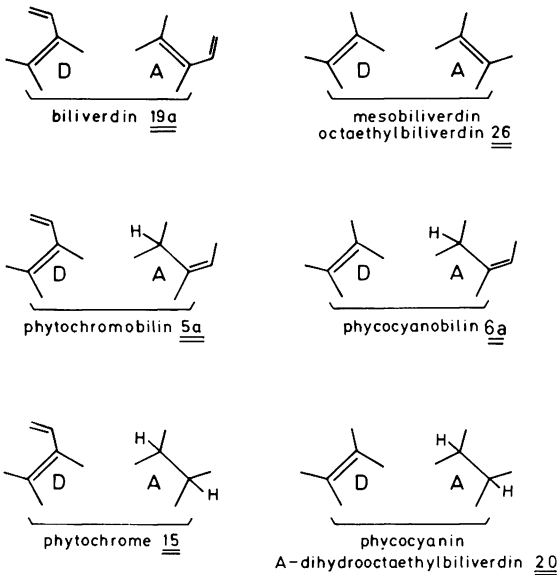
Fig. 2. Structural features of some bile pigments and biliprotein chromophores related to phytochrome P_r. Only substituents of rings A and D are given as relevant for spectral properties, all saturated substituents are indicated by a single line. Rings B/C and connection between all 4 rings are identical [see formula (5)] except for the octaethyl-derivatives bearing ethyl groups at all eight β-pyrrolic positions

A comparison of phytochromobilin and phytochrome (Table 1) reveals a spectral shift which is due to the ethylidene group at ring A in the former pigment. This group is absent in phytochrome (see Fig. 2). The same spectral

shift is also observed with phycocyanobilin and PC (Table 1). Apparently, the ethylidene groups of the free bile pigments are absent as long as the pigments are covalently linked to the protein. Therefore the ethylidene side chain of ring A has been deduced as the site of linkage with the protein.

2.1.3 Cleavage from the Protein

The successful cleavage of the covalent linkage between bile pigments and proteins in plant biliproteins was a precondition for the elucidation of the structures of the free bile pigments. The first method applied to PC and PE, namely treatment with cold concentrated HCl (O'HEOCHA 1963, O'CARRA et al. 1964) was abandoned later because it can yield artifactual bile pigments (BEUHLER et al. 1976). The second method, cleavage with boiling methanol (O'CARRA and O'HEOCHA 1966) and higher alcohols (FU et al. 1979) led to isolation and structural elucidation of phycocyanobilin and phycoerythrobilin (CRESPI et al. 1967, COLE et al. 1967, RÜDIGER et al. 1967). However, the yield is low and possibly mixtures of isomeric bile pigments are obtained (FU et al. 1979). The best cleavage method so far which gives 100% yield of phycocyanobilin from PC is the cleavage with HBr in trifluoroacetic acid (KROES 1970, SCHRAM and KROES 1971). This method also cleaves phycoerythrobilin from PE (BRANDL-MEIER, BLOS and RÜDIGER unpublished).

Whereas the treatment with concentrated HCl did not cleave the free chromophore from phytochrome, the method with boiling methanol was successful (SIEGELMAN et al. 1966). However, the yield was so poor that only an incomplete characterization was possible (Table 1). Also, the cleavage method with HBr in trifluoroacetic acid did not work with phytochrome (KROES 1970). This was later explained by secondary reactions of the vinyl group first with HBr and then with functional groups of the protein (BRANDLMEIER et al. 1980). The application of the HBr method to chromopeptides obtained from phytochrome yielded free phytochromobilin [(5a), see Fig. 1] and the methanol adduct (5c). Both were characterized (BRANDLMEIER et al. 1980, RÜDIGER et al. 1980; cf. Table 2) by comparison with authentic samples obtained by total synthesis (see Sect. 2.1.4).

Table 2. R_F values of bile pigments related to phytochromobilin (RÜDIGER et al. 1980). HPLC-plates (Merck, Darmstadt) coated with silica gel G, solvent A: carbon tetrachloride/ethyl acetate 1:1 (v:v), solvent B: carbon tetrachloride/acetic acid 1:1 (v:v)

	A	B
E-phytochromobilin (5a)	0.40	0.41
Z-phytochromobilin (5b)	0.45	0.48
E-phycocyanobilin (6a)	0.35	0.37
Z-phycocyanobilin (6b)	0.41	0.43
E-methanol adduct (5c)	0.27	0.35
Z-methanol adduct (5d)	0.33	0.43

2.1.4 Total Synthesis

The chemical structure of natural phytochromobilin was unequivocally confirmed by total synthesis of the racemic compound (5a) (WELLER and GOSSAUER 1980). The synthetic material furthermore allowed the investigation of the reactivity which was relevant to the cleavage reaction (RÜDIGER et al. 1980).

Important steps of the total synthesis were the connection of rings A and B, the introduction of the vinyl group at ring D and the condensation of the 2-pyrromethenone compounds (9) and (11) (rings A + B and C + D, respectively) to the final tetrapyrrole (see Fig. 3). The reaction of the monothioimide (7) (ring A) and the phosphorus ylide (8) (ring B), a general method for the synthesis of alkylidene lactams (GOSSAUER et al. 1977), had been applied before to the synthesis of phycocyanobilin (GOSSAUER and HINZE 1978) and phycoerythrobilin (GOSSAUER and WELLER 1978, GOSSAUER and KLAHR 1979). The introduction of the vinyl group starting from a primary hydroxyl function had also been applied to phycoerythrobilin (GOSSAUER and WELLER 1978). The final condensation reaction had also been applied before to a number of bile pigments. Interestingly, a photoisomerization at the ethylidene double bond of 5a was achieved (WELLER and GOSSAUER 1980). The thermodynamically more stable E-phytochromobilin (5a) was transformed into the Z-isomer (5b), which could thermally be reconverted to (5a). The analogous photoisomerization was also observed with phycocyanobilin (6a, 6b; formulas see Fig. 1).
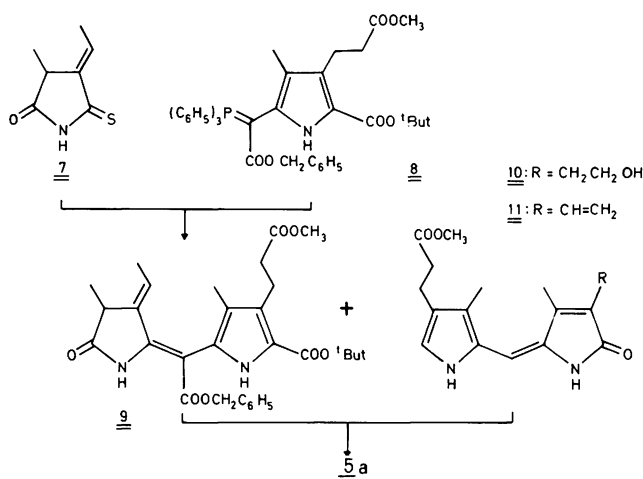
Fig. 3. Total synthesis of phytochromobilin (WELLER and GOSSAUER 1980). $tBu = C(CH_3)_3$

Treatment of E-phytochromobilin (5a) with HBr yielded a highly reactive bromo derivative which was not isolated as such. Addition of methanol led to quantitative formation of the methanol adduct (5c) (BRANDLMEIER et al. 1980). With Z-phytochromobilin (5b), the same reaction sequence yielded a mixture of (5a) and (5c) (RÜDIGER et al. 1980). Apparently, at least two reactions compete with each other, one of which finally leads to isomerization at the ethylidene group. Because some (5a) was obtained besides (5c) from the native

phytochromobilinpeptide (RÜDIGER et al. 1980), a mixture of (5a) and (5b) is considered to be the primary product of the cleavage reaction.

2.1.5 Protein Linkage and Stereochemistry

Information about the covalent linkage between phytochromobilin and the peptide moiety in phytochrome came from analysis of phytochromobilinpeptides (FRY and MUMFORD 1971, LAGARIAS and RAPOPORT 1980; see Table 3). According to this analysis, the sequence of the main product (an undecapeptide) is Leu-Arg-Ala-Pro-His-Cys-Ser-His-Leu-Gln-Tyr. Minor chromopeptides were an octapeptide and presumably a hepta- and a decapeptide derived from the same region of the peptide chain. Because the blue color was extracted at that Edman degradation step which also removed cysteine, the thiol group was considered as the chromophore-binding function of the protein (LAGARIAS and RAPOPORT 1980). This situation is the same as in PC (FRANK et al. 1978, LAGARIAS et al. 1979, WILLIAMS and GLAZER 1978, BRYANT et al. 1978) and PE (KÖST-REYES et al. 1975, MUCKLE et al. 1978, KÖST-REYES and KÖST 1979).

Table 3. Amino acid sequence analysis of a phytochromobilinpeptide. (LAGARIAS and RAPOPORT 1980)

Amino acid	Original analysis	PTH derivative recovered after each step of the Edman degradation										
		1	2	3	4	5	6	7	8	9	10	11
His	2.0					+			+			
Arg	0.9		+									
Cys	0.9						+					
Ser	0.7							+				
Gln	0.9										+	
Pro	1.0				+							
Ala	1.0			+								
Leu	2.1	+								+		
Tyr	0.8											+

The site of linkage of the thiol group was elucidated by two independent approaches.

1. It was demonstrated that synthetic thioethers, in an elimination reaction, yield different alkene compounds for different positions of the sulfur substituent (Fig. 4). The C-3 thioether (12) yields the maleimide (13) whereas the C-3^1-thioether yields the ethylidene succinimide (1a) (SCHOCH et al. 1974). Because (1a) was the only product obtained from phytochrome in this reaction (KLEIN et al. 1977; see also Sect. 2.1.1) it was concluded that the sulfur linkage is localized at C-3^1 (see partial Structure 15).

2. The same conclusion was drawn from high resolution proton NMR spectroscopy of the phytochromobilin undecapeptide (LAGARIAS and RAPOPORT 1980). This investigation was based on a previous extensive investigation of a phycocyanobilinpeptide, a synthetic reference peptide lacking the chromo-
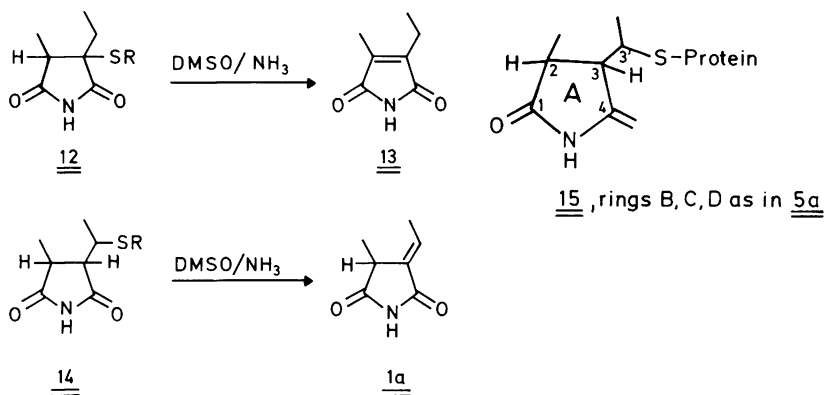
Fig. 4. Elimination reaction with synthetic thioether compounds as models for ring A of phytochromobilin. (Schoch et al. 1974)

phore and free phycocyanobilin (Lagarias et al. 1979). Double resonance experiments with the chromopeptide confirmed the hydrogenated ring A and the substitution at C-3[1]. The signals due to the chromophore in the phytochromobilinpeptide agreed well with those of the phycocyanobilinpeptide, including double resonance experiments. Therefore the structure of ring A and the thioether linkage are identical in PC and phytochrome. The only difference were the signals for the vinyl group of ring D (phytochromobilinpeptide) versus the signals for the ethyl group of ring D (phycocyanobilinpeptide).

Present knowledge on the stereochemistry of phytochromobilin and its protein linkage is only based on indirect evidence. It has been assumed that the absolute configuration at C-2 which is R in phycoerythrobilin (Gossauer and Weller 1978) and probably in phycocyanobilin (Brockmann and Knobloch 1973) is also R in phytochromobilin, but direct evidence is still lacking (Klein et al. 1977, Lagarias and Rapoport 1980). The assumption of the trans-configuration at ring A (i.e, 2R, 3R, or alternatively, 2S, 3S) was supported by the exclusive formation of trans-configurated products by addition of methanol or thiols to the ethylidene group of model imides and phycocyanobilin (Klein and Rüdiger 1978, 1979, Gossauer et al. 1980). The observed coupling constant $^3J_{2H-3H}$ in the ^{1}H-NMR spectrum of both the phycocyanobilin-peptide and the phytochromobilin-peptide agrees with the trans-configuration at ring A (Lagarias et al. 1979, Lagarias and Rapoport 1980).

Evidence for the configuration at C-3[1] came from elimination experiments (chromic acid-ammonia-degradation) in which phytochrome behaved like the model compound (16a) and differently from model compound (16b) (Klein et al. 1977). The behavior of (16a) was also found with PC and PE (Klein and Rüdiger 1978, Muckle et al. 1978) (Fig. 5). The stereochemistry of the model compounds (16a) and (16b) was unequivocally confirmed by X-ray analysis (Lotter et al. 1977, Lotter, Klein, Rüdiger unpublished). Independent X-ray analysis was performed for the corresponding imides (17a) and (17b) obtained from phycocyanobilin methanol adducts and by total synthesis (Gossauer et al. 1980).

Fig. 5. Model imides for elimination (16) and addition (17) reactions at C-3^1. The elimination was carried out with racemates, of which only one enantiomer has been drawn here. Configuration: (16a) 2R, 3R, 3^{1}R/2S, 3S, 3^{1}S; (16b) 2R, 3R, 3^{1}S/ 2S, 3S/3^{1}R. Phytochrome behaves like (16a)
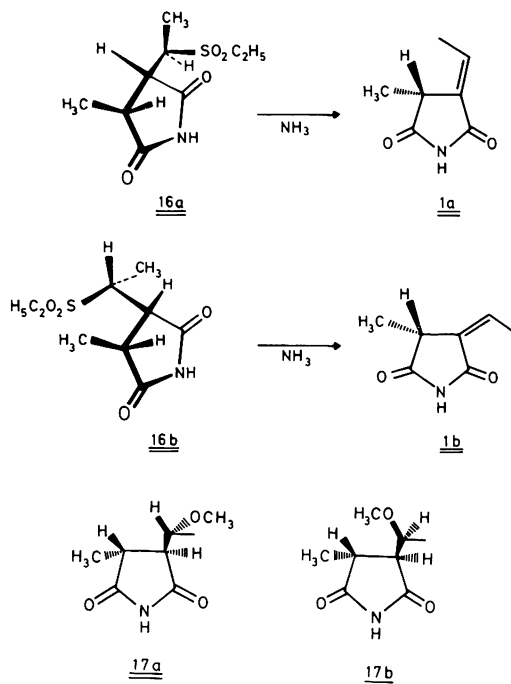
Thus the most probable configuration of the phytochrome chromophore and its protein linkage is 2R, 3R, 3^{1}R, but the alternative possibility 2S, 3S, 3^{1}S cannot yet be ruled out.

The question of a second linkage between chromophore and protein will be treated in Sect. 2.2.1.

2.1.6 The Native State

Native phytochrome (P$_r$) differs from denatured phytochrome, phytochromobilinpeptide and model compound (20) in its absorption spectrum (see Fig. 6) and many properties of the chromophore. These differences are not due to changes in the chemical structure of the chromophore and − if at all − only partly to protonation or deprotonation. They must rather be due to modification of the chromophore properties by non-covalent interactions with the native protein (see SCHEER 1980 for a discussion).

Because bile pigments are flexible molecules, the influence of chromophore conformation on spectral properties has been studied theoretically by several authors (BURKE et al. 1972, BLAUER and WAGNIÈRE 1975, SHAE and SONG 1975, SUGIMOTO et al. 1976, 1977, PASTERNAK and WAGNIÈRE 1979, SCHEER, FORMANEK and SCHNEIDER 1982). The main conclusion was that the oscillator strength of the long-wavelength band (f$_1$) is small compared to that of the short-wavelength band (f$_2$) in cyclic conformations. The predicted spectral properties were verified for biliverdin-type bile pigments with fixed extended [(25) BOIS-CHOUSSY
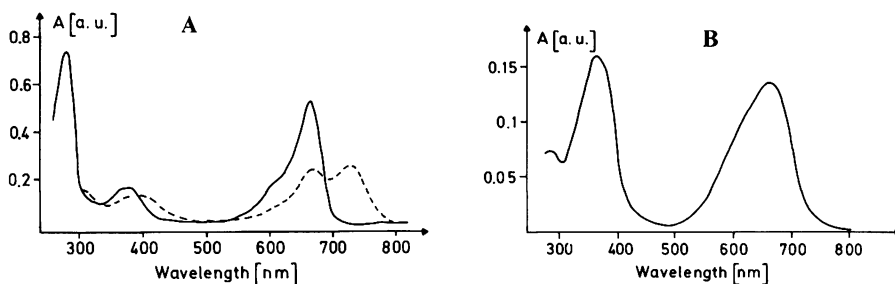
Fig. 6 A, B. Electronic spectra of phytochrome and its chromopeptide. **A** native P_r (——), native P_{fr} (------) both in 0.1 M sodium phosphate buffer, pH 7.8. **B** phytochromobilin-peptide in 10% acetic acid. (Rüdiger, Brandlmeier, Thümmler unpublished data)

and Barbier 1978)], and cyclic-helical (24) (formulas in Fig. 7) topologies (Falk and Thirring 1981), and cyclic conformations have been determined for the conformationally unrestricted pigments both in solution and in the crystal (Lehner et al. 1978a, b, Falk et al. 1978, Falk and Höllbacher 1978, Sheldrick 1976). Based on this criterion, the P_r chromophore should have a more extended conformation in its native state but a cyclic conformation in the chromopeptide and in unfolded P_r (Burke et al. 1972, Brandlmeier et al. 1981a).

Unfortunately, unfolding of P_r is irreversible. The process is fully reversible, however, with PC and PE and was investigated in more detail with these biliproteins by absorption, fluorescence, circular dichroism and chemical methods (Scheer and Kufer 1977, Langer et al. 1980, Zickendraht-Wendelstadt et al. 1980). Especially PC exhibits differences between the native and the denatured state which are very similar to those in $P_r \cdot A_2/A_1$, which is roughly proportional to f_2/f_1, increases from 0.24 to 2.32 in PC and from 0.35 to 2.27 in P_r. This indicates rather similar non-covalent interactions of the two similar chromophores (15) and (28) with the two different peptide chains of P_r and PC, respectively. The precise conformation is still unsettled. Theoretical calculations (see above) predict semi-extended to fully extended conformations. The tentative structure (15) has been proposed for the P_r chromophore in its native state because A_2/A_1 is similar to that in the fully extended isophorcabilin (25, $A_2/A_1 = 0.25$).

Ramachandran-type calculations revealed the enantioselective preference of a twisted topology for cyclic and extended conformations of A-dihydrobilin-diones (Scheer et al. 1979). These calculations agree with strong CD bands in native *and* denatured P_r (Table 5, Sect. 2.3). Interestingly, the signs of both chromophoric bands of native P_r (positive at 660 nm, negative at 365 and 377 nm) are reverted by denaturation or proteolytic digestion (negative at 665–670 nm, positive at 373–375 nm). This is in contrast to PC, in which the chromophore CD bands do not change their signs upon denaturation (Scheer et al. unpublished). The CD spectrum of denatured P_r is, however, no mirror image of that of native P_r.

Fig. 7. Proposal for the structure of native P_r chromophore, and structures for model compounds
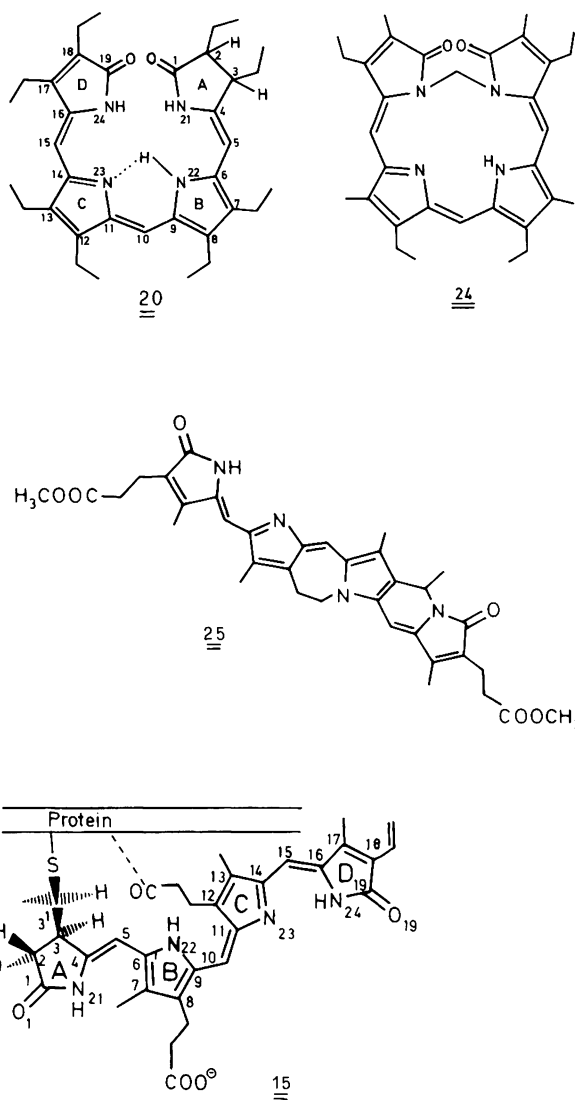
Thus the chromophores in native and denatured P_r do not represent merely an enrichment of different enantiomers, which is also likely from the different absorption spectra. Whereas a cyclic-helical conformation for the uncoupled chromophore is likely, a direct correlation of distinct conformations of the native chromophore species with its CD spectrum is not possible, but two factors relevant for the actual chromophore conformation and dissymmetry can be inferred from these experiments: The essential factors which govern the preferential chromophore helicity in P_r-peptide and denatured P_r are asymmetric centers of the chromophore itself. This influence is counteracted and overcome by the influence of the protein in native P_r.

2.2 P_{fr} Structure

2.2.1 Degradation Studies

Chromic acid degradation yields essentially the same products as obtained by degradation of P_r, irrespective of the procedure applied. Methylvinylmaleimide (4) and hematinic acid imide (2) are obtained under non-hydrolytic conditions, and additional (2) as well as methylethylidene-succinimide (1a) are liberated by subsequent hydrolysis (see Fig. 1). (1a) is also obtained by the chromic acid-ammonia method (KLEIN et al. 1977). Since the chromophore of denatured P_{fr} is stable under acidic conditions (GROMBEIN et al. 1975), a rearrangement to P_r is unlikely during degradation. It is thus concluded, that not only the β-pyrrolic substituents are the same in P_r and P_{fr}, but that also the 3^1-thioether linkage and possibly an additional ester linkage of the P_{fr} chromophore to the protein are present in both forms. Whereas any reaction of the lactam carbonyl groups (CRESPI et al. 1968, LAGARIAS and RAPOPORT 1980), Z,E-isomerizations (FALK et al. 1978), and any reaction at the α-pyrrolic and methine positions may remain unnoticed by the chromic acid degradation, the release of the SH-group with formation of an endocyclic $\Delta 2$-double bond as present in biliverdin (SIEGELMAN et al. 1968, RÜDIGER and CORRELL 1969, SONG et al. 1979, LAGARIAS and RAPOPORT 1980) or of an additional double bond at the C-3 substituent (CRESPI et al. 1968) can be excluded.

As in P_r the presence of a bond to the protein via one of the propionic acid side chains is still unsettled. Such a bond has been implicated by the release of additional (2) after hydrolysis of the chromic acid degradation products (KLEIN et al. 1977) but later been questioned as a safe criterion for such a bond in biliproteins (TROXLER et al. 1978). However, quantitative studies with radiolabelled PE from *Porphyridium cruentum* indicate a second bond in this pigment (KÖST and TROXLER unpublished). A bond of this type is absent in two chromopeptides isolated from P_r (LAGARIAS and RAPOPORT 1980), but these peptides contain a serine residue next to the binding cystein, and ester bonds are susceptible to cleavage during proteolytic digestion. A chromopeptide containing a serine-propionate bond has been isolated from PE from *Pseudanabaena* sp. W 1173, but again an artifact could not be excluded (MUCKLE et al. 1978). A definite proof may require milder degradation methods. Two such methods have been developed with PC and may be useful for phytochrome as well. The first method splits the tetrapyrrole skeleton selectively between rings A and B (SCHEER et al. 1977), the second between rings B and C. In a first application of these new degradation methods, a second protein bond at ring B in PC form *Spirulina platensis* has been suggested (KUFER et al. 1982a).

2.2.2 Spectral Studies

The spectrum of native phytochrome is shifted by approximately 70 nm ($1{,}450$ cm^{-1}) to the red upon conversion of P_r to P_{fr}. This has been taken as an indication of an increased length of the conjugated double bond system in P_{fr}, and consequently several proposals for the structure of the P_{fr} chromo-

phore are based on this interpretation (CRESPI et al. 1968, STRUCKMEIER et al. 1976, SIEGELMAN et al. 1968, RÜDIGER and CORRELL 1969, SONG et al. 1979). As has been pointed out in Section 2.1.6., however, the spectra of native biliproteins are strongly influenced by non-covalent chromophore protein interactions, which render structural assignments on the basis of the spectra of the native chromophores ambiogous (see SCHEER 1980, for references). If these interactions are uncoupled by denaturation (GROMBEIN et al. 1975) or proteolysis (BRANDL-MEIER et al. 1980, 1981a) at low pH, the 730 nm absorption of native P_{fr} is shifted to 620 nm. Denatured P_r absorbs under the same conditions at 660 nm (cation form). The P_{fr} chromophore uncoupled from the protein is stable only in its protonated form, and reverts to the P_r chromophore above pH 5 (GROM-BEIN et al. 1975). From analogy with a series of free bile pigments (KÖST et al. 1975), the free base P_{fr} chromophore can be estimated to absorb around 570 nm, corresponding to a "purpurin" (SCHEER et al. 1977) or "violin" chromophore (SCHEER and KRAUSS 1977, KRAUS and SCHEER 1979). Two conclusions can be drawn from these results: (1) The chromophores of P_r and P_{fr} are chemically different, and do not only differ by their states of protonation, or conformation (STRUCKMEIER et al. 1976; see also the theoretical studies of BURKE et al. 1972, CHAE and SONG 1975, SUGIMOTO et al. 1977, PASTERNAK and WAGNIÈRE 1979). (2) The conjugation system of the P_{fr} chromophore is one double-bond *shorter* than that of the P_r chromophore, in contrast to conclusions derived from studies of the native pigments. A chromophore structure like (18a) (Fig. 8) in which the Δ4-double-bond is (at least spectroscopically, KRAUSS et al. 1979) abolished would best agree with these data (GROMBEIN et al. 1975).

2.2.3 Chemical Model Studies

The data from chromic acid degradation and denaturation are yet insufficient to establish a complete structure for the P_{fr} chromophore. Chemical studies starting from P_r model compounds as well as MO calculations have, therefore, been carried out to give additional information on the reactivity of P_r and spectroscopic properties of chemically reasonable structures derived thereof. Possible structures for the P_{fr} chromophore obtained on this basis can then be further scrutinized by the criteria summarized below, which are derived from the known differences of P_r and P_{fr}.

1. The cation of any model compound must have an absorption around 620 nm according to the denaturation studies described in Sect. 2.2.2.
2. Denatured (GROMBEIN et al. 1975) and pepsin-digested P_{fr} (THÜMMLER et al. 1981) are convertible back to P_r both thermally and photochemically, hence any model bilin for the P_{fr} chromophore should be thermodynamically less stable than and convertible back to its original form corresponding to P_r.
3. The chromophore of native P_{fr} (see below) is probably present in its deprotonated form (PASTERNAK and WAGNIÈRE 1979, RÜDIGER 1980). In this case, the pK_B value of any model should be within the physiological pH range.
4. Both P_r and P_{fr} carry probably one single chromophore in small as well as in large phytochrome, and the molecular weight remains within the same range upon photoconversion (see PRATT 1978, RÜDIGER 1980). Any dimeriza-
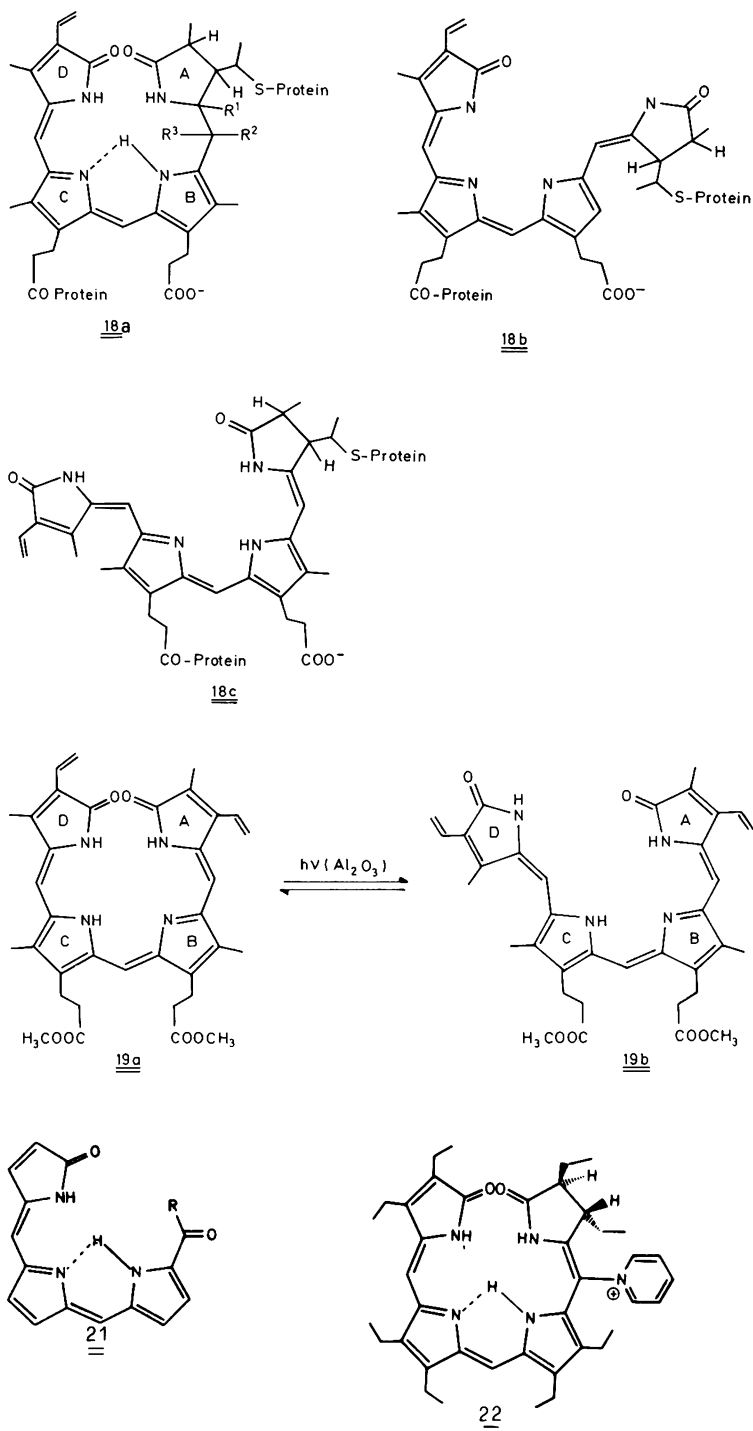
Fig. 8. Possible structures of the P_{fr} chromophore, and structures for model compounds

tion reactions of the chromophore (SCHEER and KRAUSS 1977) can, therefore, be excluded.

5. The reaction does not require any cofactors besides the protein since the phytochrome phototransformation occurs in solution of the purified pigment. Further possible criteria (e.g., stability of the P_{fr} chromophore towards reduction, oxidation, acids and bases) are discussed below.

Currently, there are two different models which meet most of the criteria summarized above.

The first model reaction is the sequence of oxidation, nucleophilic addition and tautomerization shown in Fig. 9. It is based on reactivity studies of the A-dihydro-bilindione (20), which has been taken as a model for the P_r-chromophore (SCHEER 1976). In neutral and alkaline media, (20) undergoes an easy and regioselective photooxidation at the C-5 methine bridge to a variety of products. In the presence of oxygen, "purpurins" are formed which share the oxotripyrrinone chromophore (21) (see Fig. 8) as a common substructure (SCHEER et al. 1977). In the absence of oxygen, "violins" are produced, which may all arise from one or two one-electron oxidation steps (KRAUSS and SCHEER 1979, EIVAZI et al. 1977) and a subsequent addition at C-5 (SCHEER and KRAUSS 1977, KRAUSS et al. 1979). Of particular interest is the pyridinium adduct (22) (KRAUSS et al. 1979). The spectral shift to 570 nm of the free base [criterion (1)] is − as in the E,Z,Z-biliverdin (19b) discussed below − not brought about by abolishing the $\varDelta$4-double bond, but rather by uncoupling it due to steric hindrance. The formation of (22) is thermo- but not photo-reversible [critn. (2)] and its pK for deprotonation [critn. (3)] is in the physiological region (KRAUSS et al. 1979). Criterium (4) is met as well. A reaction of this type would require very specific functions of the apoprotein as both the oxidant and the nucleophile (Fig. 9), since phytochrome is not known to contain any suitable cofactors (HUNT and PRATT 1980, QUAIL et al. 1978, ROUX et al. 1975). Cystine residues could serve as the oxidant. An involvement of cystine may be indicated by the finding of one more easily accessible SH group in P_{fr} than in P_r (HUNT and PRATT 1981). However, modification of either cysteine (HUNT and PRATT 1981) or cystine (HAHN and SONG 1981) with water-soluble reagents does not affect the phototransformation reactions. Tryptophan, tyrosin, serine, cysteine and others may serve as the nucleophile. (20) reacts readily with tryptophane derivatives to give products UV-vis spectroscopically similar to (22) (KRAUS and SCHEER 1981). In Fig. 9, tryptophane has been formulated arbitrarily as the nucleophile.

The second model is a geometric isomerization of a double bond between rings A and B or C and D. It has first been invoked by KROES (1970) and MUMFORD and JENNER (1971), but only recently gained further support. Based on earlier studies on the Z-E isomerization of dipyrroles (GOSSAUER and INHOFFEN 1970), FALK and coworkers conducted a systematic study of geometric isomerization reactions of bile pigments and partial structures derived therefrom (see FALK et al. 1978, 1980).

Biliverdin is most stable in solution in the all-syn, Z geometry (19a) (LEHNER et al. 1978, FALK and HÖLLBACHER 1978). The geometric isomers with anti-E, syn-Z, syn-Z geometry like (19b) are accessible by photoisomerization of all
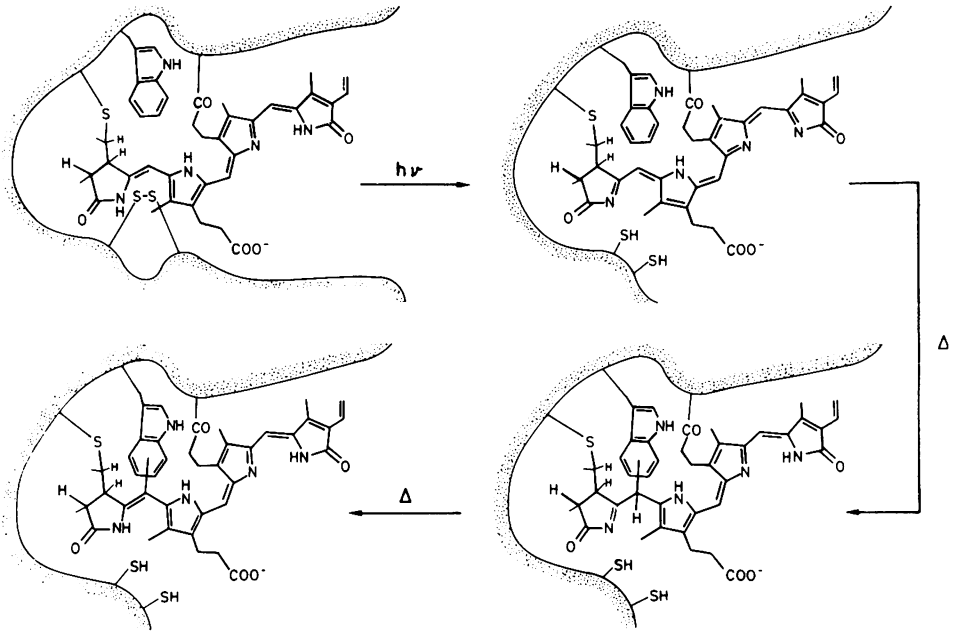
Fig. 9. Tentative model for the $P_r \rightleftharpoons P_{fr}$ interconversion as derived from photooxidations of the A-dihydrobilindion (20) as a model for the P_r-chromophore. (Krauss and Scheer 1981)

syn-Z) isomers (Falk et al. 1978) or by direct synthesis (Gossauer et al. 1981). The isomer 19b absorbs at shorter wavelengths than (19a) which is not due to the Z,E-isomerization per se but rather to a twist of the $\Delta 15$-double bond which partially uncouples ring D from the remaining π-system (Falk and Höll-bacher 1978). The isomer (19b) is thermodynamically less stable ($\Delta H^+ =$ 105 kJ mol^{-1}) and reverts to (19a) both thermally ($\Delta H^+ = 20$ kJ mol^{-1}) and photochemically (Falk and Grubmayr 1979). In biliverdins, the $\Delta 4$ and $\Delta 15$ bonds between rings A and B, and rings C and D, respectively, are very similar (Sheldrick 1976, Lehner et al. 1978b), but this is no longer true in the A-dihydropigments, of which phytochrome is a member. Ramachandran-type calculations indicate a preferential isomerization at the $\Delta 4$ bond adjacent to the reduced ring which carries the bulky thioether substituent (Scheer et al. 1979). Model compounds without this bulky substituent [e.g., (20)] yield predominantly the more stable 15E isomer (Blacha-Puller 1979, Kufer et al. 1982b). Because the UV-vis spectral properties of this 4Z, 10Z, 15E isomer are not much different from expected properties of the 4E, 10Z, 15Z compound it cannot yet be predicted whether the P_{fr}-peptide (and photoisomerized bilipeptides obtained from PC and P_r, Thümmler and Rüdiger unpublished) contains a 4E, 10Z, 15Z (18b) or a 4Z, 10Z, 15E chromophore (18c). Recent results of high-resolution NMR spectroscopy demonstrated that the P_{fr} chromophore is the 4Z, 10Z, 15E isomer 18c (Rüdiger, Thümmler, Cmiel, Schneider unpublished).
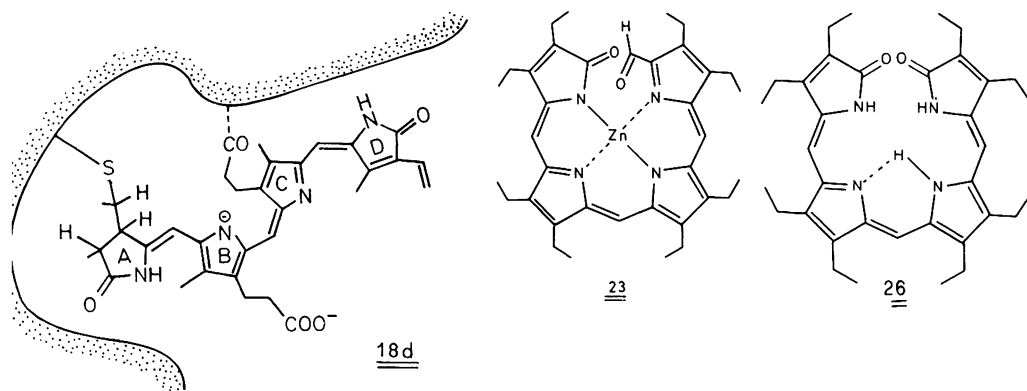
Fig. 10. Tentative structure for native P_{fr} chromophore on the basis of the Z,E-isomerization/deprotonation model

The Z,E-photoisomerization of (19) proceeds via a rubinoid pigment, which is the substrate proper for the photoisomerization (FALK et al. 1980, GOSSAUER and BLACHA-PULLER 1981). However, derivatives in which the tetrapyrrolic skeleton is strained or distorted from all-syn, Z geometry, can undergo a direct photoisomerization in solution as well (FALK and THIRRING 1979, 1980). In phytochrome, such distortions may arise both from the A-dihydrostructure and the influence of the native protein. In the P_r-peptide and in PC peptides in which a closed chromophore conformation predominates, photoisomerization so far was possible via a rubinoid intermediate. The photoisomerization product obtained in this way from the P_r-peptide is spectrally and chromatographically identically with the P_{fr}-peptide (THÜMMLER and RÜDIGER unpublished). Photoisomerization of the chromophore is, therefore, the currently most likely process during P_r–P_{fr} interconversion. It would meet the criteria (1) (2), (4), (5). The catalytic effect of redox-reagents on the P_{fr}–P_r-conversions would parallel the redox-reagent catalyzed isomerization of stilbenes (MUMFORD and JENNER 1971). A peculiar property of denatured P_{fr} and its peptides is their instability above pH 5. They are stable for hours in dilute acids, whereas the E,Z,Z-bilindiones are most stable as free bases around neutral pH. The destabilization of P_{fr} may be due to a catalytic effect of the two histidines situated next to the binding cysteine in the peptide chain (MUMFORD and JENNER 1971, LAGARIAS and RAPOPORT 1980).

2.2.4 The Native State of the Chromophore

The chromophore-protein interactions are even more pronounced in P_{fr} than in P_r (Sect. 2.1.6). The tentative structure (18d) (Scheme 9) is based on a geometric isomerization of the chromophore at the Δ4-bond discussed in the previous section, and the still rather indirect evidence presented below.

The long-wavelength absorption of native P_{fr} has its maximum at about 730 nm. Denatured P_{fr} is unstable at neutral pH, but from the absorption of the cation ($\lambda_{max} = 615$ nm), that of the free base can be estimated to $\lambda_{max} = 570$ nm (Sect. 2.2.2). This would correspond to a spectral shift of 160 nm (3,845 cm^{-1})

Table 4. Absorption maxima (λ_{max}) of and wavelength shifts ($\Delta\tilde{v}$) of different geometries and protonation states of bilindiones. The cations and anions are produced from the free bases dissolved in methanol by the addition of HCl and sodium methoxide, respectively

Pigment	λ_{max} (nm) (Form A)	λ_{max} (nm) (Form B)	$\Delta\tilde{v}$ (cm^{-1})	References
P_r	660 (native)	615 (denatured,	1,109	GROMBEIN et al. (1975)
P_{fr}	730	570 (free base)	3,845	GROMBEIN et al. (1975)
APC-I	655 (native)	600 (denatured)	1,376	CANAANI and GANTT (1980), GYSI and ZUBER (1976), ZILINSKAS et al. (1978)
APC-B	670 (native)	600 (denatured)	1,741	GLAZER and BRYANT (1975)
PC	620 (native)	600 (denatured)	537	SCHEER (1976)
PE	560 (native)	525 (denatured)	1,190	KÖST et al. (1975)
Formylbilindione zinc complex (23)[a]	830 (monomer)	750 (dimer)	1,285	STRUCKMEIER et al. (1976)
Bilindione	710 [Cyclic free base of (24)]	605 [extended, free base of (25)]	2,444	FALK and THIRRING (1981), BOIS-CHOUSSY and BARBIER (1978), BRANDL-MEIER et al. (1981 a)
Bilindione	740 [Cyclic, cation of (24)]	605 [extended, free base of (25)]	3,015	FALK and THIRRING (1980), BOIS-CHOUSSY and BARBIER (1978), BRANDL-MEIER et al. (1981 a)
Bilindione (26)[a]	657 (free base)	770 (anion)	2,234	SCHEER (1976)
A-Dihydro-bilindione (20)	594 (free base)	766 (anion)	3,780	SCHEER (1976)

[a] Formulas in Fig. 9

between the native and the denatured form. From the data in Table 4 it can
be seen that this shift is much larger than the shifts induced in free bile pigments
by conformational changes or protonation. One known process which leads
to shifts of the same magnitude is the combination of a severe conformational
change with a protonation of the chromophore [cation of the cyclic-helical
(24) vs. free base of the extended (25)]. This would require the chromophore
of denatured P_{fr} in an extended conformation, in contrast to all known free
bilindions of the violin and verdin spectral type. It would also require the chro-
mophore of native P_{fr} in a cyclic conformation, which is unlikely from a compar-
ison of native P_r and P_{fr}. Linear dichroic data indicate at most moderate geomet-
rical differences between the two forms (SONG et al. 1979). The CD bands of
P_r and P_{fr} differ in sign and magnitude (BRANDLMEIER et al. 1981b, and refer-
ences cited therein), however, and a direct comparison of P_r and P_{fr} may be
ambiguous as long as the P_{fr} structure is unknown. The other process known
to produce extreme shifts is the deprotonation of bilindiones, and especially
of the 2,3-dihydrobilindiones typical for biliproteins (SCHEER 1976). The long-
wavelength band of (20), a model for the P_r chromophore, is shifted by
$3,780 \text{ cm}^{-1}$ to the red upon deprotonation. It has been suggested on this basis
that the chromophore of native P_{fr} is present in its deprotonated form (GROM-
BEIN et al. 1975), which has been supported by molecular orbital calculations
(PASTERNAK and WAGNIÈRE 1979). This model would require a pK_B of the P_{fr}
chromophore within the physiological range leading to criterion (3) which has
been used to discriminate between P_{fr} models (Sect. 2.2.3). Protonation–depro-
tonation reactions have been suggested as primary processes in the low tempera-
ture photochemistry of biliproteins (FRIEDRICH et al. 1981a, b). Two recent
observations may also be related to a deprotonation of the P_{fr} chromophore.
The first is a pH-dependent proton uptake or release upon the irradiation of
phytochrome in solution (TOKUTOMI et al. 1982). The second is the exposure
of a hydrophobic protein surface in P_{fr} (TOKUTOMI et al. 1981, HAHN et al.
1980), which could be induced by the increased hydrophily of the chromophore.

The intensity changes of the long-wavelength absorption upon denaturation
are less pronounced than in the case of P_r (GROMBEIN et al. 1975). However,
molecular orbital calculations indicate less dramatic conformation dependencies
in the spectra of violins (PASTERNAK and WAGNIÈRE 1979). Both the red and
the blue DC bands of P_{fr} change sign upon denaturation, and the signs in
both native and denatured P_{fr} are opposite to the CD bands of P_{fr} in the respec-
tive states (BRANDLMEIER et al. 1981b; see Table 5, Sect. 2.3). This is an indepen-
dent proof of the different structures of the P_r and P_{fr} chromophores in the
denatured states. As in P_r, it is again difficult to assign a precise conformation
to P_{fr} in the native state, whereas the overriding influence of the native protein
on the chromophore is again apparent.

2.3 Phytochrome Intermediates and Modifications of the Chromophore

The phototransformations $P_r \rightarrow P_{fr}$ and $P_{fr} \rightarrow P_r$ are multistep reactions. Several
intermediates were detected by either one of the following methods: rapid kinet-
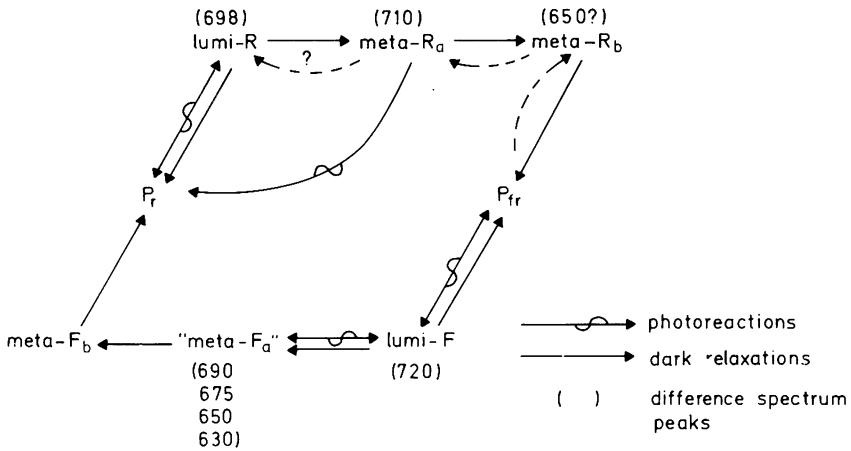
Fig. 11. Intermediates in phytochrome photoconversions including dark relaxations (*solid lines* according to KENDRICK and SPRUIT 1977; *broken lines* additional dark reactions according to Rüdiger 1980)

ics of absorption changes after flasch photolysis (LINSCHITZ et al. 1966, LINSCHITZ and KASCHE 1967, PRATT and BUTLER 1970, BRASLAVSKY et al. 1980b), low temperature spectral studies in vivo (KENDRICK and SPRUIT 1973a, b, SPRUIT and KENDRICK 1973, 1977) and in vitro (BURKE et al. 1972, CROSS et al. 1968, KROES 1970, PRATT and BUTLER 1970), dehydration in vivo (KENDRICK 1974, KENDRICK and SPRUIT 1974, SPRUIT et al. 1975) and in vitro (BALANGÉ 1974, TOBIN et al. 1973), absorption changes after continuous or during quasi-continuous irradiation ("pigment cycling", BRIGGS and FORK 1969a, b, KENDRICK and SPRUIT 1972, 1973a). Each type of study reveals the same sets of intermediates, which seem different for the forward $(P_r \rightarrow P_{fr})$ and the back reaction $(P_{fr} \rightarrow P_r)$, respectively. The subject has been reviewed by KENDRICK and SPRUIT (1977), who also suggested a nomenclature similar to the one used for the rhodopsin transformations. An alternative nomenclature based on difference maxima is included in Fig. 11.

KENDRICK and SPRUIT (1977) distinguish photoreactions and dark relaxations; the latter are further divided into those which occur in non-aqueous medium and those which require liquid water (Fig. 11).

According to KENDRICK and SPRUIT, the photoreactions (formation of lumi-R and lumi-F) and the relaxations to meta-R_a and meta-F_a are chemical events essentially restricted to the chromophore, with only minor changes of the apoprotein. These events are rapid; they occur also at low temperature and in non-aqueous medium (e.g., in dehydrated tissue in vivo, in glycerol solution in vitro). The subsequent reactions via meta-R_b and meta-F_b are believed to involve conformational interaction of the apoprotein and chromophore, since they occur only in a less rigid matrix. They are slowest in the whole reaction sequence, they require liquid water and are strongly influenced by the molecular environment.

If this view is correct, the genuine chemical differences between the P_r and the P_{fr} chromophore should already exist between lumi R or meta R_a and P_r on the one hand and between lumi F or meta F_a and P_{fr} on the other; the subsequent reaction steps (to meta R_b and P_{fr}, to meta F_b and P_r, respectively) should only serve to stabilize these differences, e.g., by conformational rearrangements. The intermediates are certainly not as well stabilized as the final products; this follows from easy back reactions (either photochemically, or chemically in the dark), of lumi R, meta R_a and possibly meta R_b to P_r, and lumi F to P_{fr} (Fig. 11). The "inverse dark reversion" of phytochrome in dehydrated tissues has been related to such a back reaction of an intermediate (KENDRICK and SPRUIT 1974) (see Chap. 17, this Vol.). The molecular basis of the presumed stabilization reaction is not clear, however. This question can be answered only after a detailed structural investigation of the intermediates, including their geometries.

A sensitive tool for conformational changes of bilin chromophores are CD spectra. The CD spectra of native and denatured forms of P_r and P_{fr} are different from each other (Table 5). Whereas the CD spectra cannot be correlated directly with defined conformations of the different forms because these are chemically different species, it is obvious that some conformational changes of the chromophore occur during $P_r \rightarrow P_{fr}$ transformation in addition to (or as a consequence of) chemical reactions. This view agrees with the observation of BURKE et al. (1972) that meta R_b (then called P_{bl}) has a low absorption but − in relation to the absorption − a strong Cotton effect of the long-wavelength band. This has been interpreted as a cyclic, possibly helical conformation for the meta R_b chromophore whereas P_r and P_{fr} should contain more extended chromophores (BURKE et al. 1972).

Table 5. Circular dichroism data of phytochrome forms. (BRANDLMEIER et al. 1981 b)

Samples	λ_{max} (nm)	$\Delta\varepsilon_{max}$ (M^{-1} cm^{-1})	Θ_{max} $\times 10^{-3}$
P_r native	660	−33.5	−110.5
	377	+35.9	+118.5
	365	+35.9	+118.5
P_r denatured[a]	670	+19.4	+64.6
	375	−14.6	−48.2
P_r-peptide[a]	665	+26.4	+87.1
	373	−15.8	−52.1
P_{fr} native (corrected)[b]	705	+8.1	
	375	−3.0	
P_{fr} denatured[a]	625	+4.75	+15.7
	380	−7.2	−23.8

[a] In 8 M urea, pH 2.5
[b] Corrected for the presence of 20% P_r

Whereas P_r is stable in the dark, P_{fr} can — at least in part — undergo a "dark reversion" to P_r. It is not yet known whether the dark reversion proceeds via similar intermediates as the photoreaction. The dark reversion depends strongly on the molecular environment. Its rate is increased by divalent metals (NEGBI et al. 1975), by pH changes and by reducing agents like sodium dithionite (ANDERSON et al. 1969; MUMFORD and JENNER 1971), as is the dark relaxation from meta R_b to P_{fr} (KENDRICK and SPRUIT 1973 a, b). Interestingly, treatment of phytochrome with dithionite leads to an addition of sulfoxylate to the chromophore (KUFER and SCHEER 1979). The equilibria between the native chromophore and the rubinoid addition products favor the native form of P_r, but the rubinoid form of P_{fr}. Thus a reaction sequence $P_{fr} \rightarrow P_{add.} \rightarrow P_r$ is one possibiltiy to explain the catalytic effect of reducing agents during dark reversion (KUFER and SCHEER 1979). Such a sequence is not feasable, however, in the acceleration of the dark relaxation from meta R_b to P_{fr}.

3 Cryptochrome

Cryptochrome, the blue light photoreceptor, has been defined by GRESSEL (1980) "as that pigment system having an action spectrum somewhat characteristic of flavins and some carotenes. This name refers to its occurrence in cryptogams and its cryptic nature." It would, of course, be a paradox in itself to deal with the chromophore(s) of a compound of "cryptic nature". However, only two candidates for the cryptochrome chromophore are earnestly being discussed, namely flavins and carotenoids with a strong preference for the former. Arguments for and against each of these candidates are discussed in an excellent and comprehensive way in the book of SENGER (1980). Only some arguments and examples can be considered here.

3.1 Flavins

The best-known flavins are FMN (27a) and FAD (27b). Both have broad absorption maxima at 370 and 450 nm, the characteristic peaks of cryptochrome action spectra. However, most action spectra show a fine structure with two additional peaks or shoulders around the 450 nm peak (see Chap. 2 and 23, this Vol.). Although this is more similar to the absorption spectra of carotenoids in this spectral region, a fine structure of the FMN or FAD band can be observed in some flavoproteins (oxidized state, GHISLA 1980) or with protein-free flavin-derivatives in extreme environment (SONG et al. 1972).

A typical reaction of flavins is their photoreduction ("photobleaching") in the presence of a suitable electron donor (e.g., EDTA). This photoreduction is also possible with flavoproteins, e.g., nitrate reductase from *Neurospora crassa* (NINNEMANN and KLEMM-WOLFGRAMM 1980). Typical is the concomitant reduction of cytochrome b-557 of this enzyme. The action spectrum for photoreduc-

tion of cytochrome b in *Neurospora crassa* in vivo had previously been shown to be the typical cryptochrome spectrum including its fine structure; interestingly the absorbance measurements of *Neurospora* cells in these experiments (prolonged irradiation) point to photobleaching of flavins without any fine structure (Muñoz and Butler 1975). This example demonstrates a principal difficulty in identifying a flavin photoreceptor: because of the high photochemical reactivity of flavins, not only the specific photoreceptor but also all or most of the flavins present in abundant amounts in the cell wall react in the same manner. The classical argument of correlation between action spectrum and absorbance or absorbance difference spectrum (upon "bleaching") is not sufficient in this case. Additional correlation arguments are needed (see Ninnemann and Klemm-Wolfgramm 1980) until the true photoreceptor and then its chromophore can be identified.
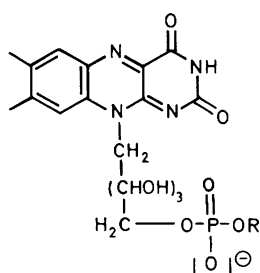
27a: R = H (FMN)
27b: R = AMP (FAD)

3.2 Carotenoids

A main argument for carotenoids as possible chromophores of cryptochrome has been the coincidence of the typical carotenoid absorption spectrum between 400 and 500 nm (including the fine structure) with the cryptochrome action spectrum. The UV absorption band which lacks in apolar solvents can be induced for carotenoids in polar solvents (Hager 1970, Song and Moore 1974). However, photoreceptor chromophores are certainly not free in solution but probably bound to a membrane or protein. Protein binding can drastically change the absorption properties of chromophores, especially if they are conformationally flexible (e.g., retinal in rhodopsin, phytochromobilin in phytochrome, see above).

It can therefore be misleading to compare the absorption of chromophore candidates in solution with the action spectrum of photoreceptors of chromoprotein nature. No defined carotenoprotein has so far been described as candidate for cryptochrome. However, an interesting model has recently been suggested by Song (1980). This is the peridinin-chlorophyll a-protein isolated from marine dinoflagellates (Fig. 12). Resonance (exciton) interactions between the carot-
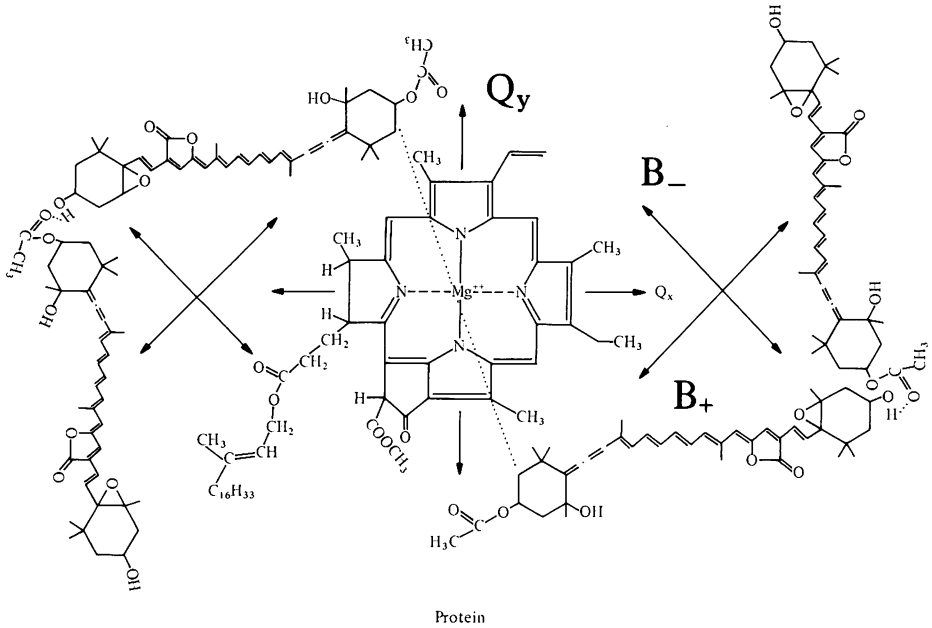
Fig. 12. The molecular topography of antenna photoreceptor complex, peridinin-chlorophyll a-protein from marine dinoflagellates. (Song 1980)

enoid molecules and between the carotenoid and the chlorophyll as acceptor molecule allows efficient energy transfer in this system. By way of energy transfer, light absorbed by the carotenoid can be active not only for photosynthesis but also for phototaxis of these organisms. A similar situation has, however, not yet been detected for cryptochrome action.

4 Phycochrome, Phycomorphochromes and Adaptachromes

In many cyanobacteria and red algae, light-stimulated developmental responses (e.g., chromatic adaptation, induction of filamentous growth) have been observed which suggest the presence of photoreversibly photochromic pigments as photoreceptors (see Bogorad 1975, Björn and Björn 1980) (Table 6). They have been termed phycochromes (in analogy to phytochrome), but as long as a correlation of distinct pigments with any of these responses is lacking the response oriented terms adaptachromes and phycomorphochromes (Bogorad 1975) are recommended (see Björn and Björn 1980 for a discussion). The action spectra of the responses show a red-green photoreversibility (Table 6). It should be noted that red-green photoreversible morphogenic effects were also described in higher plants (Klein 1979).

From the shape and the maxima of the action spectra, biliproteins have been implicated as receptor pigments. Scheibe (1972) first isolated a biliprotein

Table 6. Action maxima for photoreversible photoresponses in blue green algae correlated to phycomorphochromes and adaptochromes, for (partially) purified phycochromes and for reversible absorption changes in partially denatured phycobiliproteins

	"Green" form		"Red" form		References
	λ_{max}	Action	λ_{max}	Action	
Tolypothrix tenuis, *Fremyella diplosiphon*	540–550 350–387	PE formation	641–660 360–463	PC formation	FUJITA and HATTORI (1962), DIAKOFF and SCHEIBE (1973), OHKI and FUJITA (1978), VOGELMANN and SCHEIBE (1978), HAURY and BOGORAD (1977)
Nostoc muscorum, *N. commune*	520–550	Reversion	640–650	Induction of filamentous growth	LAZAROFF and SCHIFF (1962), ROBINSON and MILLER (1970)
Synechocystis sp. 6701	540	PE formation	640	Reversion	TANDEAU DE MARSAC et al. (1980)
"Scheibe's pigment"	520	Formation P_{650}	650	Reversion	SCHEIBE (1972), OHKI and FUJITA (1979a)
Phycochrome a	630	Formation P_{580}	580	Reversion	BJÖRN and BJÖRN (1976), BJÖRN (1980a, b)
Phycochrome b	580	Formation P_{500}	500	Reversion	BJÖRN and BJÖRN (1976), BJÖRN (1979)
Phycochrome c[a]	630, 650[b]				BJÖRN and BJÖRN (1976), BJÖRN (1979)
Phycochrome d[a]	650	Formation P_{650}	610–620	Reversion	BJÖRN (1978, 1980, personal communication)
APC	645	Formation P_{555}	560	Reversion	OHAD et al. (1980)
PC, 0.5 M guanidinium chloride	570	Formation P_{630}	630	Reversion	OHKI and FUJITA (1979b)
APC, 0.5 M guanidinium chloride	600	Formation P_{650}	650	Reversion	OHKI and FUJITA (1979b)
APC, pH <4	645	Formation P_{555}	550–560	Reversion	OHAD et al. (1979, 1980)

[a] A correlation has recently been indicated between the occurence of phycochrome b and d and the presence of phycoerythrocyanin in the respective organism (BJÖRN 1979, 1980b)

[b] Probably two different forms

fraction from photobleached *Tolypothrix tenuis,* which gave photoreversible absorption difference spectra reminescent of the action spectra for chromatic adaptation 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 and BJÖRN 1976, BJÖRN 1978, 1979) (Table 6). All appear to be biliproteins, but the purified fractions generally exhibit only absorption differences which do not exceed a few percent. A notable exception is phycochrome b in which absorption differences of about one third of its maximum absorption allowed an in vivo detection (BJÖRN 1979). To establish the identity of any such isolated fraction with a photoreceptor proper, 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).

28
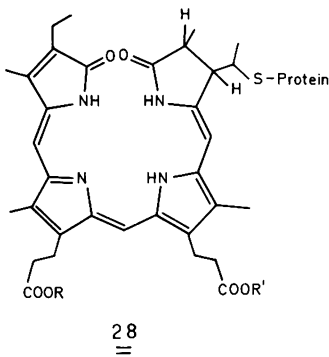
The need for a distinction becomes even more obvious from recent results on the photochemical properties of phycobiliproteins partially transformed in vivo (OHKI and FUJITA 1979a) or partially denatured in vitro (OHKI and FUJITA 1979b, OHAD et al. 1979, 1980) (Table 6). By this treatment, APC and PC containing the chromophore (28) obtain photoreversibly photochromic properties reminescent to the phycochromes. Especially the results with the isolated and purified biliproteins indicate that photochromicity is an inherent property of the bulk biliproteins and not related to a co-isolated impurity. The induced photochromicity of PC and APC decreases again at more severe denaturation conditions. Possibly, the "tickling" of the protein loosens 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. 2.1.6 and 2.2.4) then favors the latter processes. Stepwise denaturation has been observed with PC from *Spirulina platensis,* suggesting a distinct intermediate in the unfolding process (SCHEER and KUFER 1977). Similar intermediates may be present in the pigments isolated from *T. tenuis* (OHKI and FUJITA 1979b) and *F. diplosiphon* (OHAD et al. 1979, 1980). In summary, the function of phycochromes as photoreceptors remains doubtful and needs further evaluation.

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