Photosynthetic Light-Harvesting Systems

Organization and Function

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PHOTOCHROMIC PROPERTIES OF C-PHYCOCYANIN

G.Schmidt, S.Siebzehnrübl, R.Fischer, and H.Scheer
Botanisches Institut der Universität, Menzinger Str. 67,
D-8000 München 19, F.R.G.

Summary

This contribution describes work aimed at understanding the different properties of the structurally very similar chromophores of the antenna pigment, C-phycocyanin (PC) from Mastigocladus laminosus, and of the photomorphogenetic reaction center pigment, phytochrome. In particular, the ZZZ-ZZE photoconversion of PC chromophores was explored. Native PC is unreactive except for a slow, irreversible bleaching. It attains photoreversibly photochromic properties upon partial uncoupling of the chromophores by certain perturbation of the protein structure. Two treatments are particulary effective: a) Dehydration and b) partial denaturation with mercaptoethanol. The results show that relatively small changes of the apoprotein structure are sufficient to induce an efficient photochemistry in PC, which is reminescent of the phytochrome primary reactions.

Introduction

The use of certain cofactors for a variety of functions is a common mechanism of adaptation on the molecular level for which the term "molecular ecology" seems appropriate. The plant biliproteins are an example for such adaptations. Phycobiliproteins, the photosynthetic antenna pigments of cyanobacteria, red and cryptophyte algae and the photomorphogenetic receptor pigments, phytochrome and phycochrome(s), bear very

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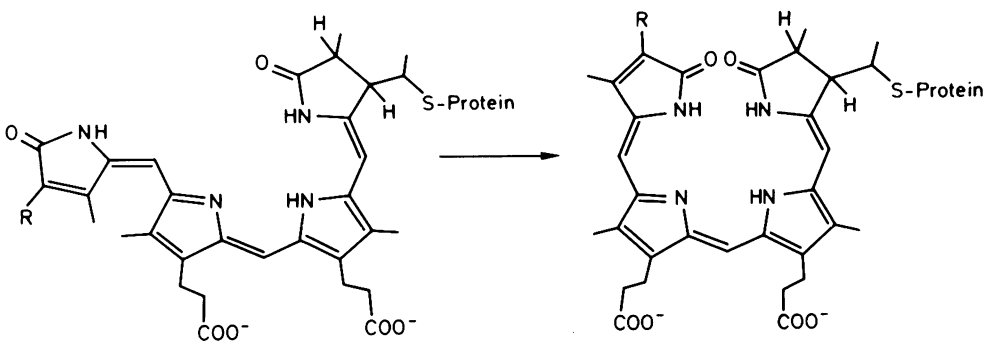
similar open-chain tetrapyrrolic chromophores (1). I.e, the chromophores of phycocyanins (PC) and allophycocyanins (APC) and the chromophore of phytochrome in the P_r-form, differ only by the substituent at C-18. In addition to these similarities in molecular structure, their covalent bonds to and their non-covalent interactions with the apoproteins are very similar as evidenced by their spectroscopic properties (2).

None the less are the functions of these pigments in vivo and many properties in the isolated states very similar for the two pigment classes. Phycobiliproteins like PC are chemically and photochemically inert. They are highly fluorescent in the isolated state and transfer excitation energy efficiently to the reaction centers in the native environment (1). Phytochrome is by contrast photochemically very reactive, and shows accordingly only little fluorescence. Its primary photochemistry is the reversible ZZZ-ZZE interconversion of the chromophore (3), (Fig. 1). It should be noted, that the properties of both pigments in the native state are drastically different from those of the free chromophores or denatured biliproteins, which are photolabile, chemically reactive and have very different conformations.



Phycocyanin R = C_2H_5
Phytochrom R = C_2H_3

In this contribution, we wish to present results which explore the possibility of photochromic reactions, similar to those of phytochrome, in the phycobiliproteins. There is a large body of data which relates the operationally defined phycochromes, e.g. photomorphogenetic receptor pigments of cyanobacteria, to subunits of the "common" antenna biliproteins (4,5). Partially denatured biliproteins are known to exhibit small, albeit distinct photoreversibly photochromic responses, which have in one case been related to a distinct chromophore (6). One of the chromophores in PC from two different species, e.g. ß-155, has also been shown by x-ray crystal structure analysis to be present in a strongly twisted conformation in which ring D is nearly perpendicular to the other rings (7). This has been related to the presence of small amounts of ZZE-chromophores being present in PC from Mastigocladus laminosus after denaturation (8). The large variations in the amount of ZZE chromophores prompted us to conduct a systematic search for the conditions under which ZZE chromophores are formed, and to study their reactions in the native and denatured state of the protein. Information on photoreactivity is also important with respect to applications of laser spectroscopic techniques to phycobiliproteins.

Materials and Methods

PC was prepared from frozen cells of Mastigocladus laminosus as previously described (9). Subunits were prepared by isoelectric focussing in a flat-bed aparatus on dextran gel. The separation was carried out in denatured state (7M urea) under argon and in the dark. After elution from the gel, the subunits were renatured by dialysis against 0.1M K-phosphate buffer pH=7, first at room temperature and then at 4C. Further purification was done by two steps of a ammonium sulphate precipitation (45%).

PC and subunits with ZZE-chromophores were prepared in 8M urea buffer, containing 0.1M K-phosphate, pH=7. The solution was treated in the dark with 15 vol% mercaptoethanol and then irradiated in an ice bath under nitrogen for ten minutes with white light. The following steps were carried out under green safety light. After elimination of the thiol at pH=2 (addition of hydrochloric acid), the equilibrium mixture of ZZZ/ZZE-products was cleaned up in two steps with a desalting gel to remove mercaptoethanol and to keep the photoisomerisation products in acidic denatured state, or in neutral K-phosphate buffer. The content of modified ZZE-chromophores was determined again by saturating irradiation with white light. Determinations of yield were based on the difference spectrum of ZZE/ZZZ interconversion of a pure ZZE PC-chromopeptide (3).

Absorption difference spectra were recorded with a ZWS II - spectrophotometer (Biochem, Puchheim) in split beam mode with thermostated (12C) cell holders. One of the cuvettes can be irradiated in the photometer by a Lumilux 150 Watt light source (Volpi, Denzlingen). The light guide from light source to the sample can be equipped with suitable interference filters. Analog data were digitized with a Technosystem (Darmstadt) model 1203A A- to -D converter and fed to a microcomputer (Apple IIe). Difference spectra against the unirradiated sample were recorded before and after irradiation.

Results and Discussion

Assay for presence of ZZE-configurated chromophores

PC and its subunits containing a part of the chromophores in the ZZE configuration (table 1) were prepared from native PC by a sequence of reactions which are well established. They involve denaturation of the protein, addition of thiols at C-10, irradiation, elimination of thiol and renaturation. The

15E-isomer is formed regioselectively, if this same sequence, with the omission of protein denaturation/renaturation steps, are applied to A-dihydrobilins (10). The ratio of ZZZ-to-ZZE in the product mixture was determined by denaturation of the modified pigment in acidic urea and illumination with white light to saturation. Under these conditions, the ZZE form is quantitatively converted to ZZZ (11), and the amplitude of the difference spectrum (δA, minimum - to - maximum, see fig.1) is proportional to the amount of ZZE in the original mixture [from the differential extinction coefficient $\delta\varepsilon$=35100, taken from the spectra of PC-peptides (3) and the molar extinction coefficient of the denatured ZZZ-chromophore cation of PC ε=30300 (12); this amount can be calculated by the equation $Q_{ZZE} = 86 * \delta A / A$)]. The same assay has been used throughout the further studies, and the occurence of such light induced difference spectra has been taken as evidence for a ZZE-ZZZ conversion.

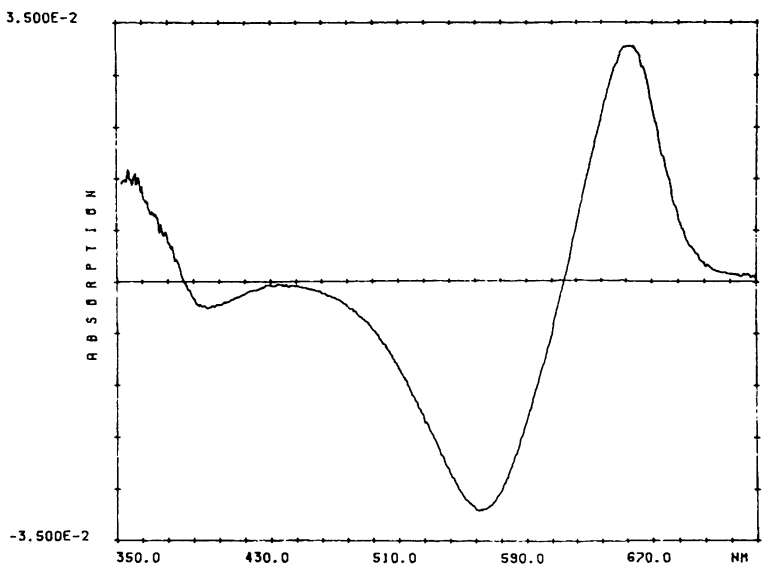


Fig. 1: ZZZ-ZZE difference spectrum of PC after denaturation (8M urea, 0.1M K-phosphate, pH=1.9) and irradiation with white light to saturation

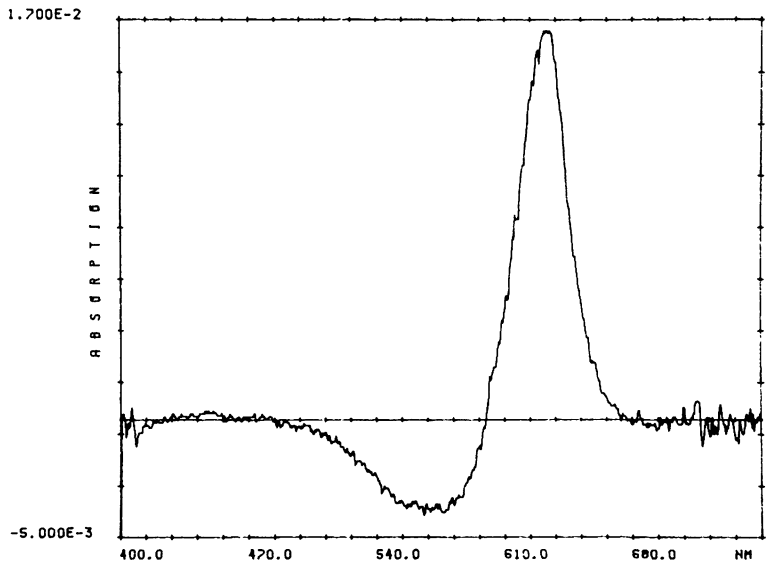
Table 1: Photoisomerisation of ZZE-PC and its Subunits to ZZZ in Denatured State (8M Urea, 0.1M K-Phosphate, pH=1.9) by Irradiation with White Light (60/120sec).

ZZE- preparation-method	sample	content of ZZE-isomers (%)
denatured, via C-10 rubinoid addition product (see text)	PC	12.0
	α-subunit	15.5
	β-subunit	9.5
partially denatured (4M urea, 0.1M K-phosphate, pH=7.1, irradiation: 180sec at 630nm)	PC	2.4
	α-subunit	-
	β-subunit	5.9
partially denatured (0.1M K-phosphate, pH=7.4, 20vol% mercaptoethanol, irradiation: 300sec at 600nm)	α-subunit	13.8
	β-subunit	60.2

Photoconversion of ZZE-chromophores in native protein

To obtain the ZZZ/ZZE difference spectrum of a chromophore in a quasi-native environment, the PC modified as described above was irradiated in the native state (fig.2). It is dominated by a broad decrease in absorption at 575 nm, and a more intense, narrow-banded absorption increase around 630 nm. Even after saturating irradiation, only part (appx.30 - 40%) of the ZZE chromophores are phototransformed as evidenced by the further transformation after denaturation. This indicates a differential reactivity of the chromophores in the native protein, by steric hindrance or the like.

Fig. 2: ZZZ-ZZE difference spectrum of PC chromophores with native apoprotein (0.1M K-phosphate. pH=7). The spectrum has been obtained after saturating irradiation with white light.



PC is photochromic in the dehydrated state

By measuring the light induced difference spectra obtained after acid urea denaturation of different PC preparations, it had been observed that many PC-samples, contained small and varying amounts of ZZE chromophores. A systematic investigation showed dehydration to be most effective in inducing the formation of ZZE chromophores, and that this formation is light dependent (table 2). ZZE chromophores were found in particular in samples which had undergone a lyophilisation process in the light. There is no detectable ZZE content if freshly isolated PC is lyophilised in the dark, whereas irradiation during lyophilisation or of the lyophilised material resulted in ZZE contents up to 5%. One possibility for photo-

isomerisation would be the formation of rubinoid addition products at C-10, which are known to photoisomerise (see previous section). Several amino acid side chains would principally allow such a reaction. However, this possibility is ruled out by monochromatic irradiation. Red light absorbed by the PC chromophore is more effective than blue light (table 2). Together with the results of the previous section, this indicates that PC becomes photochromic in the dehydrated state.
Small amounts of ZZE chromophores were also present in the isolated, not lyophilised, subunits. The factors leading to their formation were not further investigated, but they are formed most likely in the denaturation-renaturation cycle at intermediate urea concentrations. A complete shielding from light during the preparation is very difficult, and a light-

Table 2: Photoisomerisation of PC and its β-Subunit during Dehydration (Lyophilisation)

irradiation of PC during lyophilisation at nm:	content of ZZE-isomers %
dark control	0.3
370	-
430	2.4
530	3.4
630	4.6
660	3.8
white light (400-700)	5.3
irradiation of the β-subunit during lyophilisation at nm:	
dark control	1.7
white light (400-700)	5.2

induced isomerisation similar to the one described for partially denatured PC (6) may well be responsible.

PC is photochromic upon partial denaturation of the apoprotein

Various denaturing agents including acid (13), guanidinium chloride (14) and ethylene glycol (15) have been shown earlier to induce photochromic properties of PC chromophores. In a previous study with urea (6), it could be shown that "tickling" with urea resulted in a specific activation of only one of its three chromophores, e.g. β-84. The maximum amount of ZZE remained well below 10% in all previous studies, however. In an extension of the work with ethylene glycol, the thiol analogue mercaptoethanol now proved much more efficient (table 1).

This was originally a chance - observation during attempts to omit urea from the preparation of thiol adducts at C-10. If large amounts of mercaptoethanol (≥25 vol%) are added to native PC, the rubinoid addition products are formed nearly quantitatively even in the absence of any urea. Titration with the reagent clearly indicated that this addition proceeds in two steps. The first one is a denaturation of the protein, as evidenced by the decrease of the 620nm absorption of the chromophores, and an increase around 350nm. This is followed by the nucleophilic addition of mercaptoethanol at C-10 and the concomitant absorption increase around 415nm. It was thus not surprising to find ZZE chromophores in preparations of PC, subjected to treatment with mercaptoethanol and subsequent irradiation, since the photoreactivity of bilirubins is well documented (11,16). The amounts of ZZE chromophores in such preparations were unusually large, however, and they peaked under conditions where part of the phycocyanobilin chromophores were still unreacted with the reagent.

To further investigate this reaction, mercaptoethanol was added to the β-subunit of PC in an amount sufficient to trans-

form appx.50% of the chromophores to the yellow addition product. This mixture was then subjected to irradiation with light. The absorption at 604nm decreased rapidly with only little change in other regions of the spectrum above 300nm (fig. 3). An analysis of the mixture gave a ZZE yield of 60%,

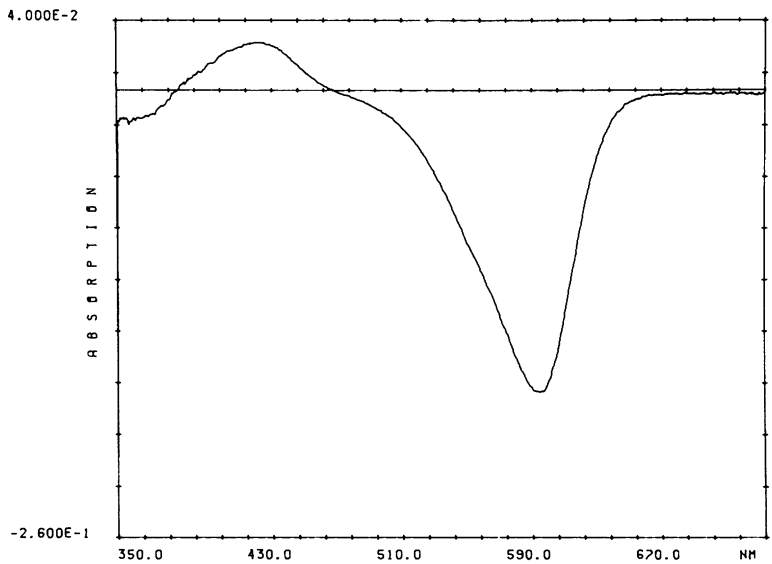


Fig. 3: Photoreaction of PC in the presence of mercaptoethanol (20%) with white light. The absorption at 620nm corresponds to the phycocyanobilin chromophore partially uncoupled from the apoprotein, that around 425nm to its addition product with mercaptoethanol.

which is four times the amount found after irradiation of the rubinoid pigment. Monochromatic irradiation furthermore gave maximum yields of ZZE chromophores with red light, absorbed by the PC chromophore, rather than with blue light absorbed by the thiol addition product (table 1). This shows clearly, that the photoreaction is not due to the well known isomerization of the yellow addition product, but rather to a very efficient

photochemistry of the phycocyanobilin chomophore itself. It is noteworhy, that the proportion of ZZE is much larger in thr β- than in the α-subunit. This is similar to the reactivity in the present of 4M urea (6) and indicates a selective activity of (one of) the β-chromophores.

Conclusions

Photoreversibly photochromic properties can be induced in PC by several kinds of perturbation of the protein structure. The reactions involve most likely Z-E interconversions of the chromophores at the 15,16 double-bond between rings C and D. This reactivity is reminescent of the primary photochemistry of phytochrome. There is a differential reactivity of the chromophores.

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References

1. Scheer, H.. 1982. In: Molecular Biology. Biochemistry and Biophysics, Vol 35 (F.K. Fong, ed.). Springer-Verlag Berlin, pp. 7-45.

2. Rüdiger, W., H. Scheer. 1983. In: Encyclopedia of Plant Physiology, New Series, Vol. 16 (W. Shropshire, Jr., H. Mohr, eds.). Springer-Verlag Berlin.

3. Thümmler, F., W. Rüdiger. 1981. Tetrahedron 39, 1943-1951.

4. Björn, L.O. 1979. Quart. Rev. Biophys. 12, 1-23.

5. Kufer, W. this book.

6. John, W., R. Fischer, S. Siebzehnrübl, H. Scheer. 1985. In: Antennas and Reaction Centers of Photosynthetic Bacteria: Structure, Interaction and Dynamics (M.E. Michel-Beyerle, ed.), Springer Verlag Berlin, pp.17-35.

7. Schirmer, T. 1987. J. Mol.Biol. 196, 677-695.

8. Schmidt, G., S. Siebzehnrübl, R. Fischer, H. Scheer, T. Schirmer, W. Bode, R. Huber. 1987. Z. Naturforsch. 42c, 845-848.

9. Füglistaller, P., H. Widmer, G. Frank, H. Zuber. 1981. Arch. Microbiol. 129, 268-274.

10. Kufer, W., H. Scheer. 1982. Z. Naturforsch. 37c, 179-192.

11. Falk, H., K. Grubmayr. 1979. Monatshefte für Chemie 110, 1237-1242.

12. Kufer, W., H. Scheer. 1981. Hoppe-Seyler's Z. Physiol. Chem. 360, 935-956.

13. Ohad, I., H.-J. Schneider, S. Gendel, L. Bogorad. 1980. Plant Physiol. 65, 6-12.

14. Ohki, K., Y. Fujita. 1979. Plant Cell Physiol. 20, 483-490.

15. de Kok, J. 1983. Private publication.

16. Mc Donagh, A., D. Lightner, T. Wooldridge. 1979. Journ. of the Chem. Soc., 109-112.