

**POZNAŃ TECHNICAL UNIVERSITY
POLISH BIOPHYSICAL SOCIETY**

**PHOTOBIOLOGY
AND
BIOTECHNOLOGY**

**PROCEEDINGS
OF INTERNATIONAL SYMPOSIUM**

**JUNE 27-30 · 1989
POZNAŃ · POLAND**



UER028023126158

THE INTERNATIONAL SYMPOSIUM ON
"PHOTOBIOLOGY AND BIOTECHNOLOGY"

Poznań, Poland, June 27–30, 1989

Is organized by:

Institute of Physics, Poznań Technical University,
Institute of Environmental Engineering, Poznań Technical
University,
Institute of Commodity Science, Academy of Economics in
Poznań.

Under the auspices of the Polish Biophysical Society, Polish
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Wydanie I. Nakład 520+30 egz. Arkuszy wyd. 10,7. Arkuszy druku 11,75. Papier offsetowy kl. III 71 g. Przyjęto do druku 13.02.1989 r. Podpisano do druku 22.03.1989 r. Druk ukończono w kwietniu 1989 r. Zamówienie nr S/74/89.K-8/114

Wykonano w Zakładzie Graficznym Politechniki Poznańskiej
61-821 Poznań, ul. Ogrodowa 11, telefon 525-425

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Phycobiliproteins: Photochemistry and Photophysics

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Introduction

The common phycobiliproteins (Phycocyanin; PC, allophycocyanin; APC, phycoerythrin; PE, and phycoerythrocyanin, PEC) are photosynthetic light-harvesting pigments in cyanobacteria, rhodophytes and cryptophytes (Scheer, 1982). Special (pools of) phycobiliproteins termed adaptochromes and photomorphochromes, have also been implicated as sensory photoreceptors in cyanobacteria (Bogorad, 1975), similar to the phytochromes of plants and algae (Rüdiger and Scheer, 1985). Photoreversibly photochromic biliproteins have subsequently been isolated from several species, but their relations to the antenna and/or photomorphogenetic pigments remain unclear (Björn and Björn, 1980).

During studies on phycobiliproteins from cyanobacteria, we became interested in the factors regulating the contributions of fluorescence, photochemistry and radiationless decay to excited-state relaxation. Particular emphasis was placed on the aggregation state and on defined structural modifications of these pigments. This work was undertaken to complement time-resolved studies where the potential buildup of background at high repetition rates can lead to problems, and to yield more information on the possible involvement of phycobiliproteins in light perception. Here, we wish to present results obtained with two phycobiliproteins, PC and PEC from the thermophilic cyanobacterium, *Mastigocladus laminosus*, for which primary (Zuber, 1986), crystal (Schirmer et al., 1987; Düring and Huber, private communication, 1988) and chromophore structures (Scheer, 1982; Bishop et al., 1987) are known. Experimental work on energy transfer carried out in cooperation with the group of Siegfried Schneider (Technical University, Muenchen, FRG; Schneider et al., 1988), is presented separately in the same volume.

Photochemistry

Long-lived photoproducts have been observed in phycobiliproteins under a

variety of conditions. The most interesting and potentially most important type is a photoreversible photoreaction which appears to be very similar to the reaction of phytochrome. Compared to the latter, the difference extrema in the optical spectra are blue-shifted. They occur in the orange-red and green spectral region, whereas the difference extrema of phytochrome are in the red and far-red.

In the phytochrome, the Z/E-isomerization of the chromophore is the basic photoreaction, as has been shown by ¹H-nmr spectroscopy of chromopeptides isolated from the different forms, P₁ and P₂ (Rüdiger *et al.*, 1984). The 15E-isomer is the one absorbing at longer wavelengths (\approx 730 nm), the 15Z-isomer absorbs at \approx 660nm. The latter is more stable, and synthesized in the dark.

PC is photochemically inactive in its native state, except for a slow and irreversible bleaching (Scheer, 1987). Under the conditions isolated (pH 7.5, 50-100 mM phosphate buffer), it is free of linkers and occurs mainly as a trimer ($\alpha_3\beta_3$). Photoreversible photochromism is observed under a variety of conditions, which all involve partial dissociation and/or denaturation of the polypeptide. Difference extrema are in the 650 and 570 nm range. The magnitude of the photoreaction is generally expressed in $\Delta\Delta A$ -units, e.g. as the minimum-to-maximum amplitude of the reversible difference spectrum. This is similar to phytochrome research, but the values were here normalized to the maximum absorption in the red. The magnitude is only a few percent in the presence of moderate amounts of urea (Schmidt *et al.*, 1988), ethylene glycol (DeKök, 1984), propylene glycol (Murakami and Fujita, 1983) or the like. Small effects are also observed in lyophilized PC (Schmidt *et al.*, 1988). The magnitude can be as high as 60%, however, in the presence of 20% mercaptoethanol (Schmidt *et al.*, 1988).

The molecular basis is still not known, but likely to involve also a Z/E interconversion of the chromophore(s) at the $\Delta_{15,16}$ double-bond. Partly E-configured PC can be prepared semi-synthetically from the native Z-PC *via* the rubinoid reduction product (Schmidt *et al.*, 1988). It shows only irreversible photochemistry in the native (pH 7.5, phosphate buffer) and fully denatured state (pH 1.5, 8 M urea). The difference spectrum obtained after irradiation with visible light is identical, however, to that of the photoproducts of PC brought to the same conditions.

Under the aforementioned conditions, one or more of the chromophores of PC are apparently capable to perform the same type of photochemistry as the one of phytochrome. A major difference is, however, that in the latter the 15E-isomer

is the one absorbing at longer wavelengths, whereas it is the 15Z-isomer in PC. A special type of protein-chromophore interaction has been postulated for phytochrome to account for the unusual red-shift, which is obviously absent in PC.

More recently, we have studied in cooperation with W. Kufer from our laboratory a different pigment, e.g. PEC. It is a relatively rare biliprotein replacing phycoerythrin in some cyanobacteria (Bryant, 1982). Its structure is very similar to that of PC (Zuber, 1986; Düring and Huber, private communication), but it contains an unusual phycoviolobilin chromophore instead of the phycocyanobilin chromophore at cystein-84 of the α -subunit (Bishop *et al.*, 1987).

Being a component of the phycobilisome, it is commonly regarded a light-harvesting pigment. However, its α -subunit had been linked previously to photochromic activities in cyanobacterial extracts, and possibly to photomorphogenesis (Björn and Björn, 1980; Kufer, 1988). Phycochrome b is most likely α -PEC. Both the α -subunit and integral PEC show photoreversible photochromism, whereas the β -subunit is inactive or shows only irreversible photochemistry. Difference extrema for the former are around 600 and 500 nm.

A distinct difference from PC, is that substantial photochemistry occurs in PEC at all states from native through fully denatured. If this reaction involves also a Z/E isomerization (which is indicated from several lines of evidence including optical and IR-difference spectra), it would require two factors: One is a changed reactivity of the chromophore, which has to be active in its free form uncoupled from the protein. Phycocyanobilin is inactive under such conditions. The other is a decreased rigidity in the environment of the α -84 phycoviolobilin chromophore. This was indeed born out in the crystal structure of PEC (Düring and Huber, private communication).

Effect of Chromophore Structure on Phycobiliprotein Aggregation

The effect of disaggregating agents to induce photochemistry in PC has been discussed above. In PEC, there is likewise an increase of photoactivity in the presence of such reagents, e.g. the amplitude of the difference spectrum is maximum at 4-5 M urea. The potentially more interesting reverse effect has now been observed for PEC. This pigment shows a reversible effect of photochemistry on its aggregation. When PEC is alternately irradiated with orange (600nm) and green light (500nm), there occurs at the same time a photoreversible change in aggregation: The amount of trimer increased each

time the last irradiation was with green light, and decreased each time it was with orange light. This means, that the configuration of chromophore α -84 influences aggregation. The effect can be rationalized again from the x-ray structure: α -84 is located very close to the contact surface of interacting monomers in trimers.

To test the sensitivity of aggregation to the structure of α -84 chromophores, we have also performed experiments with PC. The chromophores of integral PC were reduced to rubins by treatment with NaBH. This modified pigment was monomeric when analyzed by ultracentrifugation, but became trimeric again after reoxidation. In a second series of experiments, isolated subunits were reduced to rubins. Modified α -subunits were then hybridized with original β -subunits and vice versa, to yield hybrid PC. Again, the hybrids containing modified subunits formed monomers, whereas after reoxidation of the chromophores they reaggregated to trimers.

These results point to an involvement of the biliprotein chromophores not only in energy transfer and photochemistry of biliproteins, but also in their structure. In the context of their possible function as sensory pigments, a dissociation of biliproteins could be the origin of a signal chain leading eventually to photomorphogenesis. These results do not prove an involvement of PC or PEC in light perception of cyanobacteria, but they show at least a possible mechanism by which chromophore photochemistry can reversibly modulate the biophysical properties of these proteins, and by which proteins as small as the phycobiliproteins could be involved in more than one function.

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