PHOTOBIOLOGY
The Science and Its Applications

Edited by

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UV Light and DNA Damage, Repair and Mutagenesis

Photochemistry and Photophysics of Biliprotein Chromophores: A Case of Molecular Ecology

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Biliproteins are widespread pigments in nature, and perform a variety of rather different functions (Kayser, 1985; Scheer, 1982; Braslavsky et al., 1983; Ruediger and Scheer, 1983): The phycobiliproteins occurring in cyanobacteria, rhodophytes and cryptophytes, function as photosynthetic light-harvesting pigments. Phytochromes are sensory photoreceptors in plants and algae, and the putative adaptochromes and photomorphochromes of cyanobacteria are probably also biliproteins. The bilirubin-serum albumin conjugates are involved in transporting this poorly water-soluble pigment, and many invertebrates contain biliproteins which play a role in their pigmentation, but may have additional functions.

According to these different functions, the photochemical and photophysical properties of these complexes are rather diverse; a fact that is surprising in view of the structural similarities of many of the chromophores involved. The relative contributions of different deexcitation pathways are regulated by specific interactions between the chromophores and the proteins. In analogy to interactions among living systems, I like to term these interactions as "molecular ecology".

In the context of studies on phycobiliproteins from cyanobacteria, we became interested in the factors regulating the contributions of the different deexcitation pathways, as well as in the influence of chromophore structure and photochemistry on protein structure and aggregation. Additional reasons for such studies are the potential buildup of background during time-resolved and other laser-spectroscopic techniques working at high intensities or repetition rates, and the possible involvement of phycobiliproteins in light perception (Bjoern and Bjoern, 1980; Scheer, 1982; Kufer, 1988).

Today, I would like to report some recent work we have done with two phycobiliproteins, phycocyanin and phycoerythrocyanin from the thermophilic cyanobacterium, *Mastigocladus laminosus*, for which primary (Zuber, 1986) and crystal structures (Schirmer et al., 1987; Duerring and Huber, private communication, 1988) are known.

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Energy transfer

Experimental work on this subject has been carried out in cooperation with Siegfried Schneider (Technical University, Muenchen, FRG; Schneider et al., 1988). Both pigments in different aggregate sizes, and their α- and β-subunits have been studied by two complementary picosecond techniques: The decay of fluorescence and of fluorescence polarization was measured with a repetitive streak-camera, and the ground-state de- and repopulation kinetics by absorption-recovery.

The smallest PC-unit, e.g. the monomer of the α-subunit, shows >95% of its fluorescence as a single-exponential decay with a rate constant of appx. 900 psec, an expected pattern for a single, isolated chromophore. More complex decay patterns had been observed before in subunit preparations which we now know were dimers. However, aggregation does not change the decay pattern and the depolarisation, and energy transfer among the two chromophores in the α_2-dimer appears to be negligible. The β-subunit shows a biexponential decay, with rate constants in the 30 and 1100 psec range. The first is due to energy transfer from the high-energy β-155 to the low-energy β-84, because population of the excited state of β-84 is delayed relative to the excitation of β-155 by this time. All higher aggregates require three or even more exponential components to fit the decay patterns. The most dramatic change occurs upon aggregation of the $(\alpha\beta)_1$ monomers (=heterodimers) to the $(\alpha\beta)_3$ trimers (= heterohexamers), where depolarization kinetics are increased by an order of magnitude.

In cooperation with Ken Sauer (University of California, Berkeley), we have simulated the dynamics by using several models for energy transfer, and the chromophore geometries as determined by x-ray crystallography (Sauer and Scheer, 1988). Although a model based on Foerster-type energy transfer gives results which model satisfactorily existing experimental data, there is evidence that excitonic coupling cannot be ignored in trimers and higher aggregates. α-84 on one monomeric unit, and β-84 on the adjacent one, come so close that Foerster transfer times <1 psec are calculated, and excitonic coupling energies in the range of $75 cm^{-1}$. This intermediate region between Foerster transfer and excitonic interaction is hitherto only little explored, and the phycobiliproteins may be useful in this respect.

Radiationless deactivation

The single-exponential lifetime of the α-subunit is in the range of 900 psec, whereas the longest fluorescence component in larger aggregates (assigned to cumulative decay of all chromophores over which excitation energy is thermally equilibrated) is 1.4–2 nsec. Since oscillator strengths of the chromophores do not change markedly upon aggregation of the phycobiliproteins, the increase in fluorescence lifetimes then corresponds to a decrease in energy losses with increasing aggregate size. This corroborates earlier steady-state results showing an increase in fluorescence yields from around 40% in subunits to close to 90% in aggregates.

As photochemistry to stable products is no major deexcitation pathway in PC (see below), and intersystem crossing is not important either, these losses are due to efficient internal conversion. Three mechanisms to this have been discussed in free bile pigments (Scheer, 1982, Braslavsky et al., 1983). The first is a high density of vibrational states, which is related to the high conformational flexibility of bile pigments. The second deexcitation mechanism is *via* photochemical channels leading to the ground-state of unstable products, which revert to the ground state of the original species. Specifically, internal H-transfer and Z/E-isomerizations at the central methine bridge (C-10/11) have been discussed. Isomerization involves rather large structural changes which are unlikely in the native or near-to-native protein environment. H-transfer is, on the other hand, even a candidate for photochemistry at temperatures close to absolute zero (Koehler et al., 1988). Little is known on the flexibility of the chromophores in their native environment, but reagents known to increase protein mobility decrease biliprotein fluorescence. Obviously, the details of radiationless deexcitation need further study.

Photochemistry

Whereas the photochemical events discussed in the context of radiationless decay are transitory, long-lived photoproducts have been observed in several phycobiliproteins. The most interesting and potentially most important type of photochemistry is observed upon partial denaturation. This photochemistry has now been found to occur under a variety of conditions. Its magnitude (defined by the ratio of the amplitude of the difference spectrum, to the maximum absorption) can be as high as 60% in the presence of 20% mercaptoethanol. It is most likely due to a Z/E interconversion of the chromophore(s) at the C-15,16 double-bond. Under such conditions, the chromophores are apparently capable to perform the same type of photochemistry as that of phytochrome.

More recently, we have studied in cooperation with W. Kufer from our laboratory a different pigment, e.g. PEC. Its α-subunit had been linked previously to photochromic activities in cyanobacterial extracts, and possibly to photomorphogenesis (Bjoern and Bjoern, 1980; Kufer, 1988). This pigment, which is structurally very similar to PC (Bryant, 1982; Duerring and Huber, private comm.), carrries a rare phycoviolobilin chromophore at cys α-84 (Bishop et al., 1987), which replaces the common phycocyanobilin chromophore present at the same location in PC. Being a component of the phycobilisome, it is commonly regarded a light-harvesting pigment. A distinct difference from other phycobiliproteins, is however its pronounced photochemistry in the native state, which is most likely again a Z/E-isomerization at the C-15 methine bridge. Th reaction would require a decreased rigidity in the environment of α-84, which has been born out in the crystal structure of PEC (Duerring and Huber, private comm.).

Effect of chromophore α-84 on aggregation

Recently, we noticed that PEC shows not only increased photochemistry upon disaggregation, but there is also a reciprocal dependence of biliprotein aggregation on photochemistry. When PEC is alternately irradiated with orange (600nm) and green light

(500nm), the two photoequilibria were enriched in the 15-E- and 15-Z-configured forms, of α-84 chromophore respectively. Ultracentrifugation showed, that at the same time there occurs 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 α-84 controls aggregation. This can be rationalized again from the X-ray structure: α-84 is located very close to the contact surface of monomers in trimers.

To test the sensitivity of aggregation to the structure of α-84 we have done another experiment with PC. The chromophores in isolated subunits were reduced to rubins. Modified α-subunits were then hybridized with original β-subunits and *vice versa* to yield hybrid PC. In this experiment, the hybrids containing modified α-subunit only formed monomers, whereas those hybrids containing modified β-subunits reaggregated to trimers.

This result points to an involvement of the biliprotein chromophores not only in energy transfer and photochemistry of biliproteins, but also in their structure. This effect may well be at the origin of a signal chain leading eventually to photomorphogenesis. In a more general context, it is an example for the intricate interplay of proteins with their cofactors, which leads to the stunning variety of properties of pigments with the same or very similar molecular structures.

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