Chapter 4

PHOTOCHEMISTRY OF PHYCOERYTHROCYANIN
LITERATURE REVIEW:

The photochromic (photoreversible) pigments regulating morphogenesis, mobility and pigment synthesis in various cyanobacterial species were reviewed by Lazaroff (1973) and Bogorad (1975). These photochromic pigments resemble the phytochrome of higher plants but have their absorption peaks at lower wavelengths which have been termed as phycochromes. Scheibe in 1972 first isolated a biliprotein fraction from photobleached Tolypothrix tenuis which gave photoreversible absorption difference spectra reminiscent of the action spectra for chromatic adaptation in this species. Bjorn in 1976 investigated the photochromic responses of pigments from cyanobacteria. They fractionated the aqueous extracts of different species of cyanobacteria by electrofocussing. In all the algae investigated, fractions with isoelectric points at or near 4.6 showed photochromic behaviour analogous to that of phytochrome in higher plants although they were sensitive to light at shorter wavelengths. They classified them into three main types: phycochromes a, b and c. Phycochrome a has one form absorbing maximally at 590 nm (formed under red light) and the other form at about 630 nm (formed under green light). Phycochrome b has one form absorbing maximally near 510 nm.
and the other form at 570 nm (formed in yellow green light and blue green light respectively). Phycochrome c has one form absorbing maximally at 650 nm (formed under green light) and the one absorbing weakly in the green region (formed under red light). Phycochrome c is less sensitive than phycochromes a and b. During the same time they have also presented evidence that one type of photochromic change observed in fractions obtained from several blue-green algae correspond to transforming of s-type chromophores into f-type chromophores in green light and vice versa in red light. They examined the transformation kinetics and quantum yield and also produced evidence for other types of photochromic pigments from blue-green algae. Bjorn (1978) reported phycochrome d, a new photochromic pigment from blue-green alga *Tolypothrix distorta*. Bjorn in 1979 reviewed the green/red antagonism in blue-green algae marked by photoreversibility which resembles red/far red antagonism of phytochrome regulated processes in higher plants. After the characterisation of different phycochromes by Bjorns the need for establishing the identity of any such isolated photochromic pigments with a photoreceptor proper was necessary to confirm the information obtained by spectral data. Ohad et al. in 1979 studied the effect of temperature on the forward and backward reaction of the photoreversion
process. Contemporary to the findings of Bjorn and Bjorn 1976, Bryant et al. (1976) were involved in discovering the new biliprotein phycoerythrocyanin (PEC). They have purified PEC from two species of cyanobacteria. Okhi and Fujitha (1979a) studied the photochemical properties of phycobiliproteins partially transformed in vivo. Mac Coll et al (1981) studied the spectroscopic behaviour and properties of phycoerythrocyanin from Anabaena variabilis in whole cells and isolated PBSomes. They have extended the work to Mastigocladus laminosus. The studies of Okhi and Fujitha 1979b and Ohad et al 1979 and 1980 indicated the APC and PC containing the chromophore, 28 (c.f. Scheer, 1983) obtain photoreversibly photochromic properties reminiscent 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 severed uncoupling then favours
the latter processes. Okhi and Fujitha (1979b) reported that purified C-PC and APC from *Tolypothrix tenuis*, which were not photoreactive in buffer solution, showed small but distinct photoreversible reactions after partial denaturation with guanidine-HCl or urea or after incubation of the protein extracts in phosphate buffer of alkaline pH. de Kok et al (1981) induced photoreversible reactions in C-phycocyanin from *Anacystis nidulans* by dissolving ethyleneglycol to a high concentration of 75% (v/v). Irradiation with red light (638 nm) causes a 7.5% decrease in absorbance around the absorption maximum (620 nm) while the absorbance around 500 nm increases. Subsequent irradiation with green light (500 nm) partially reverses this change. Final photoreversibility at around 620 nm amounts to ca. 2.5% of maximum absorbance. These reactions were ascribed to two interconvertible species PCr and PCg, the former with a higher absorbance in the red and the latter in the green. The rate of dark reversion from PCg to PCr is strongly enhanced by ferricyanide. It is proposed that with this reagent, dark reversion occurs via an oxidised form of PCg. Furthermore ferricyanide in the presence of ethyleneglycol is capable of reversibly oxidising part of chromophores of C-phycocyanin, presumably to a radical. In the absence of ethyleneglycol, however
ferricyanide causes total irreversible bleaching of the pigment in dark. The induced photoreversibility of C-phycocyanin is ascribed to the perturbing action on the protein structure by ethylene glycol in high concentrations. This solvent proved to be the most suitable perturbant of several compounds tested. Scheer in 1983 reviewed briefly about the phycochrome, phycomorphochrome and adaptochromes. Bjorn et al (1983) described the photochromic pigments in akinetes in comparison with vegetative cells of Anabaena variabilis Kutzing. They have made a search for the phycochromes b and d in the akinetes of Anabaena variabilis. The different facts obtained by isoelectric focussing were investigated for the presence of phycochroms b and d by measuring the absorption difference spectra. Phycochrome b was also assayed for by measuring in vivo absorption difference spectra. The assays were positive for all three pigments. Bjorn et al in 1984 studied the light induced linear dichroism by plane polarised light in photoreversibly photochromic pigments. They found out that the transition moment of chromophore has the same direction with respect to the protein in the long wavelength and the short wavelength forms of phycochrome b. Phycochrome b (Bjorn and Bjorn 1976, Bjorn 1979, 1980) is a photochromic
pigment, present in vegetative cells of PEC containing blue green algae like *Tolypothrix distorta* and a species of *Anabaena variabilis*. It is also present in akinetes of *Anabaena variabilis* (Bjorn et al 1983) and may be involved in light induction of their germination (Bjorn and Bjorn 1981). With blue-green and yellow green light it can be converted into forms with absorption maximum at 500 and 570 nm, respectively.

Kufer and Bjorn in 1989 separated the biliprotein PEC into its α and β subunits from the cyanobacteria *Mastigocladus laminosus* Cohn and *Tolypothrix distorta* Kutzing var. *sympliocoides* Hansgirg, strain IUCC 424 (now UTEX 424). In both the cases the isolated α-subunits showed the photoreversible photochemistry characterising phycochrome b, a photoreversibly photochromic pigment so far found only in extracts of PEC containing organisms. They have also studied the light induced absorbance changes in β-subunit and in phycoerythrocyanin. Siebzehnrubl et al. (1989) have recently studied the reciprocity of reversible photochemistry and aggregation in phycoerythrocyanin from *Mastigocladus laminosus*. They reported the photoreactivity observed under different conditions from highly aggregated to fully denatured PEC, the reactivity of the subunits under
similar conditions, and the mutual interdependence of phototransformation and aggregation. They visualised the decrease in the photochemistry of larger aggregates. *Siezebehnrubl et al. (1990)* made a comparative study of PEC from two species of cyanobacteria, *Mastigocladus laminosus* and *Chroococcidiopsis sp.*

**RESULTS:**

All photochemical studies on PEC were performed with various preparations ranging from intact PBSomes to PEC in different forms. In all the cases the photochemistry was induced as mentioned in materials and methods. The intact phycobilisomes showed little photochemistry. The intact phycobilisomes were dissociated in potassium phosphate buffer (5mM, pH 7.0) at 4°C overnight. After monitoring the extent of dissociation by the absorption and fluorescence emission, the dissociated PBSomes were subjected to photochemical induction. The light induced absorption changes in dissociated PBSomes are shown in fig. 4.1. Fig. 4.2 deals with the absorption difference spectra of dissociated PBSomes fig. 4.1. Trace 1 of fig. 4.2 shows extrema at 502 nm (positive) and 567 nm (negative). The photochemistry induced by orange light in dissociated PBSomes is totally reversed by green light (fig. 4.2, trace
Fig. 4.1 Light quality induced absorption changes in dissociated phycobilisomes from Westiellopsis prolifica. (---) After 10 min saturated pre-irradiation with 500 nm light and (-----) subsequent irradiation with 600 nm light for 8 min.
fig. 4.1
Fig.4.2 Absorption difference spectra of dissociated phycobilisomes

Trace 1. Absorption difference spectrum of 8 min 600 nm illumination minus 10 min 500 nm pre-irradiation.

Trace 2. 8 min 600 nm illumination followed by 10 min 500 nm minus 10 min 500 nm pre-irradiation.

Trace 3. Spectra as seen for intact phycobilisomes after 8 min 600 nm illumination
fig. 4.2
Fig.4.3 Light quality induced absorption changes in PEC (x)

Trace 1. Absorption spectra of PEC (x) after
saturating pre-irradiation with 600 nm
light for 8 min.

Trace 2. Subsequent saturating irradiation with
500 nm light for 10 min.
fig. 4-3
PEC (x), the first fractions obtained on passing dissociated PBSomes on DEAE cellulose columns showed an intense absorption change after eight minutes of 600 nm illumination with a 500 nm pre-irradiation of 10 minutes (fig. 32). Figure 33 deals with the changes in absorption monitored simultaneously with an illumination of 600 nm light for ten minutes and the spectra recorded after every ten seconds time interval. Fig. 4.4. confirms the absorption changes of PEC (x) through difference spectra. Trace 2 of Fig. 4.4 reveals that the induced photochemistry of PEC (x) is totally reversible. The next fractions to be examined for photochemical changes were PEC trimers. Fig. 4.6 and 4.7 deal with the organe light induced changes in absorption and the difference spectra of the same PEC trimers. Again the photochemistry was totally reversible upon post illumination with green light (fig. 4.7 trace 2). Addition of KSCN to 1M to PEC trimers yields PEC monomers. PEC monomers differ slightly as far as the orange light induced absorption changes are concerned. There is pronounced bleaching of $\beta$-unit which is prominent in absorption spectra (fig. 4.8) and absorption difference spectra (fig. 4.9). Fig. 4.9 trace 2 deals with total reversibility of the photochemical reaction with post
Fig. 4.4 Absorption difference spectrum of
Trace 1. Trace 1 minus trace 2 of Fig. 4.3.
Trace 2. Difference spectrum (8 min 600 nm
followed by 10 min 500 nm minus 10 min
500 nm pre-irradiation
Fig. 4.5 Absorption spectra of PEC (x) recorded after 10 min green irradiation and subsequent illumination with organe light. Spectra were recorded at an interval of 10 sec during the illumination of sample with organe light.
Fig. 4.6 Light quality induced absorption changes in PEC trimers.

(-----) Absorption spectra of PEC trimers after saturating pre-irradiation with 600 nm light and (---------------) subsequent saturating irradiation at 500 nm.
Fig. 4.7 Absorption difference spectra of PEC trimers.

Trace 1. Absorption spectra after saturating pre-irradiation with 600 nm light minus absorption spectra of subsequent illumination at 500 nm.

Trace 2. Difference spectrum of 8 min 600 nm followed by 10 min 500 nm minus 10 min 500 nm light.
fig. 4.7 Wavelength, (nm)
Fig. 4.8 Light quality induced absorption changes in PEC monomers.

(----) Absorption spectra of PEC monomers after saturating pre-irradiation with 600 nm light for 8 min and subsequent saturating irradiation at 500 nm (-----).
Fig. 4.9 Absorption difference spectra of PEC monomers

Trace 1. Absorption difference spectrum of 8 min 600 nm illumination minus 10 min 500 nm pre-irradiation.

Trace 2. 8 min 600 nm illumination followed by 10 min 500 nm preirradiation minus 10 min 500 nm pre-irradiation.
fig. 4.9
WAVELENGTH (nm)
irradiation of 10 minutes with green light although the PCB chromophore in the subunit showed considerable bleaching.

DISCUSSION:

In intact phycobilisomes from Westiellopsis prolifica no extrema was observed around 503-571nm in the difference spectrum during the attempted induction of photochemistry in these systems. (Fig 4.2 trace) In these PBSomes, a fluorescence emission maximum around 680nm indicated the perfect intactness and efficient energy transfer to the terminal emitters. Although negligible, Siebzehnrubl et al, (1989) detected a photochemistry of 0.31-0.36% with extrema at 502 and 567nm in the difference spectrum obtained for two preparations of intact PBSomes. They have indicated a partial loss of terminal emitters in their PBSome preparations with an emission maximum at 665 nm and a shoulder at 683nm. They suggested two reasons for decreased photochemistry in higher aggregates. The first reason could be due to a reduced photoreactivity by a decreased mobility of the \(\alpha\)-84 PXB chromophore (fig. 4.10) which inhibits the rotation of ring D necessary for isomerisation. \(\alpha\)-84 chromophores of PEC to be more photochemically active need increased mobility of the PXB chromophore. This enhances
Structures of phycoviolobilin chromophores in Z,Z and Z,E-configuration.
the rotation of ring D necessary for isomerisation. Thus, in intact phycobilisomes due to the compact packing of all the chromophores, the photochemistry was not seen in our case. The second reason attributed to the low photochemistry was that the decreased photochemistry may also be due to a competition of energy transfer to lower energy phycocyanobilin chromophores with photochemistry. This can again be true in intact PBSomes where the energy transfer is highly efficient and occurs at a picosecond time scale to the terminal pigments wherein they emit at a higher wavelength of 680 nm and photochemistry if at all present goes undetected. It is not so the case with dissociated PBSomes. Phycoerythrocyanin is known to dissociate more readily than the other biliproteins (Bryant, 1982). This enables the increased mobility of α-84 chromophores which enhance the rotation of ring D necessary for isomerisation. There is also less competition of photochemistry with energy transfer to lower energy phycocyanobilin chromophores which is evident from the fluorescence emission spectrum of dissociated PBSomes. Here, the PEC shows an emission peak at 580 nm indicating the loss of energy transfer from PEC--PC. The two reasons attributed by Siebzehnrubl et al. (1989) are responsible for the increased photochemistry in dissociated PBSomes. Thus, we attribute both the reasons to
our studies with regard to the photochemistry of PEC in intact and dissociated PBSomes. We conclude that in dissociated PBSomes PEC is more mobile wherein the energy transfer to PC is inhibited due to which photochemistry is observed. The PEC trimers registered a similar percentage of photochemistry as observed by Siebzehnrubl et al. 1989 for *Mastigocladus laminosus*. In the case of monomers we again see a photochemistry as high as 41% which is 5% more than that observed for their counterparts from *Mastigocladus laminosus*. The patterns of changes observed in absorption spectra and absorption difference spectra were again similar to those of Siebzehnrubl et al. (1989). Here again when we look at the fluorescence emission spectra of PEC trimers and PEC monomers, we find a difference in the energy transfer patterns. In trimers a single emission peak at 630nm indicates a perfect energy transfer from $\alpha$-PXB to $\beta$-PCB. But two emission peaks in the case of monomers confirm the loss of energy transfer from $\alpha$-PXB to $\beta$-PCB. Under such conditions an increase of photochemistry in monomers again may be attributed to high mobility of $\alpha$-84 chromophores due to loss of energy transfer from $\alpha$-PXB to $\beta$-PCB when compared to PEC trimers. Coming to the PEC(x) fractions we observed an intense photochemistry of about 84% which is higher than any
Table: 4.1

Photochemistry of phycoerythrocyanin from *Westiellopsis prolifica* in various aggregation states

\[ \Delta \Delta A = \frac{\Delta A_{\text{orange}} - \Delta A_{\text{green}}}{A_{\text{green}} \text{ max}} \times 100\% \]

<table>
<thead>
<tr>
<th>Example</th>
<th>Concentration of potassium phosphate buffer, pH 7.0</th>
<th>Difference spectra, (nm)</th>
<th>( \Delta \Delta A ) (%)</th>
<th>Reversibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact PESomes</td>
<td>1M</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dissociated PESomes</td>
<td>5mM</td>
<td>567</td>
<td>503</td>
<td>6.1</td>
</tr>
<tr>
<td>FEC (x)</td>
<td>50mM</td>
<td>570</td>
<td>508</td>
<td>84.8</td>
</tr>
<tr>
<td>FEC</td>
<td>100mM</td>
<td>570</td>
<td>508</td>
<td>17</td>
</tr>
<tr>
<td>FEC and KSCN to 1M</td>
<td>567</td>
<td>505</td>
<td>41</td>
<td>Total</td>
</tr>
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other form of native PEC reported so far. Even the \( \alpha \)-subunit from *M. laminosus* (Siebzehnrubl et al., 1989) registered a photochemistry of 50%.

By our studies we conclude that photochemistry is the highest in lowest aggregates ranging from PEC(x) to intact PBSomes. Further based on the photochemistry observed in dissociated PBSomes, PEC monomer and PEC(x), we are of the opinion that PEC in *Westiellopsis prolifica* is more photoreactive when compared to its counter part in *Mastigocladus laminosus*. We also add that in all the cases the \( \alpha \)-84 chromophores are responsible for the reversible photochemistry. An irreversible bleaching in the PC absorption region observed in PEC monomers is due to \(-PCB\) chromophores. All our data from *Westiellopsis prolifica* support the observations of Siebzehnrubl et al. (1989) with regard to *Mastigocladus laminosus*.