

Photosynthetic Light-Harvesting Systems Organization and Function

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C-PHYCOCYANIN FROM MASTIGOCLADUS LAMINOSUS: CHROMOPHORE
ASSIGNMENT IN HIGHER AGGREGATES BY CYSTEIN MODIFICATION

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The phycobilisome (PBS) is a unique antenna system, because well-defined building blocs can be isolated from it, which allow principally to study the functional properties, e.g. light absorption and excitation energy transfer, as a function of aggregate size and functional complexity (1-4). Besides the so-called linker polypeptides, it is mainly composed of phycobiliproteins (phycoerythrin (PE) or phycoerythrocyanin (PEC), phycocyanin (PC) and allophycocyanin (APC)). Each phycobiliprotein is composed of two (or sometimes more) subunits (α, β) bearing 1-4 open-chain tetrapyrrolic chromophores, which are covalently bound to cystein residues of the apoprotein via thioether linkages (3-5). In any given phycobiliprotein the chromophores differ in their spectroscopic properties and their chemical reactivities. As shown in Table 1 for C-phycocyanin (PC) from Mastigocladus laminosus discussed here, this is even true for pigments bearing chromophores of identical molecular structure. These differences arise from the different proteinenvironment and distinct conformations of the individual chromophores in the native state.

The polypeptide and chromophore structures of tri- and hexamers of C-phycocyanins (PC) which are the basic building blocs of phycobilisomes, have been determined with high resolution by x-ray cristallography (5). For the analysis of energy transfer

(6,7) and photochemical properties (8) it is necessary to know in addition the electronic structures (9) and component spectra (6,10) of the individual chromophores. The component spectrum for the single chromophore of PC (α -84, Fig.1) can be determined directly from the isolated α -subunit. The ones of the two β -chromophores (Fig. 1) have been obtained by resolution of the absorption spectrum of the β -subunit (6,10) and assignment of the β -84 chromophore by modification of the neighboring cys-111 with p-chloro-mercury benzenesulfonate (PCMS) (11). The same components can be used for the (α,β)-monomer (=heterodimer), because its spectrum is the sum of the subunit spectra.

Table 1 : Specific Attributes of Individual Chromophore Types of PC from Mastigocladus laminosus (from ref. 3,4,6,8,10,11)

Characteristics	α - 84	β - 84	β - 155
Reaction with PCMS	-	+	-
Absorption Maximum [nm]	616 - 618	622 - 624	598 - 600
Fluorescence	+	+	-
Optical Activity	+	-	+
Reversible Photochemistry	-	+	+/- ⁽¹⁾
Conformation According to X-Ray Crystal Structure	ZZZ	ZZZ	ZZX ⁽²⁾

- 1) The contribution of this chromophore to photochemistry is still uncertain.
- 2) Ring D is almost perpendicular to ring C (5, see also 8)

The spectroscopic properties of larger aggregates can no longer be described as a sum of the same subunit component spectra (Table 2). The absorption maxima of higher aggregates show more or less pronounced red-shifts which have to be accounted for in the component spectra. This is in particular necessary with linker-containing trimers and phycobilisomes.

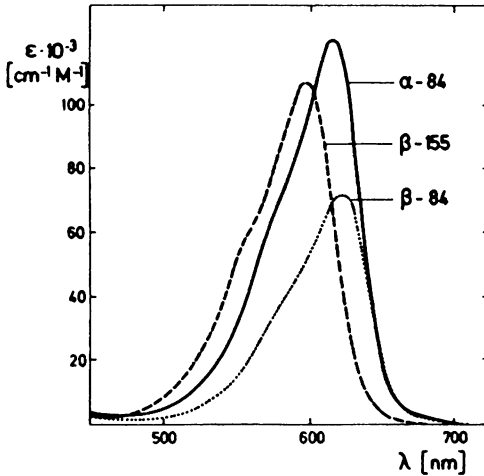


Fig. 1: Single chromophore spectra of PC from M. laminosus. The spectra of chromophores β -84 and β -155 were obtained by deconvolution of the β -subunit spectrum assuming a similar lineshape as the α -84 chromophore.

Table 2 : Some Characteristics of PC in Different Aggregation States and its Two Subunits

Unit	Aggregation state of PC	$n^1)$	Absorption λ_{max} [nm] [cm ⁻¹ mM ⁻¹]		Molecular weight [kDa]
α -Subunit	dimer	2	616	244	36.0 ²⁾
β -Subunit	dimer	4	604	328	38.8 ²⁾
PC (α, β)	monomer	3	612	286	37.4 ²⁾
PC (α, β) ₃	trimer	9	619	896	112.2 ²⁾
PC+linker	trimer	9	630	nd	≈140
PBS	≥dodecamer ³⁾	≥36	≈632	-	≈560

1) n = number of chromophores

2) according to amino acid sequence (12)

3) calculated per rod, each phycobilisome contains six rods

Component spectra thus have to be derived separately in such aggregates. It has been suggested, that interactions of the linkers with chromophore β -84 may be an important factor in spectral modifications, because it is close to the central cavity of the doughnut-shaped trimers and hexamers in which the linkers are probably located (5,13).

To test the hypothesis, we have extended the PCMS method to tag this chromophore in phycocyanins from different origins and of different aggregation states. The results (Table 3) show that it is possible by this method to obtain perturbation spectra related to chromophore β -84 of PC in aggregates ranging from the β -subunit to PBS (APC is unreactive, and the PEC difference spectrum occurs at much shorter wavelengths than that of PC).

Table 3 : Treatment of PC-Aggregates and -Subunits with PCMS

Pigment	Absorption λ_{max} [nm]	Absorption difference				$t_{1/2}$ 2) [min]
		λ_{min} [nm]	% 1)	λ_{max} [nm]	% 1)	
<u>M. laminosus</u>						
PC (monomer)	612	613	14.3	654	9.1	15
PC (trimer)	618	626	17.6	658	16.1	120
α -Subunit	616	-	-	-	-	-
β -Subunit	604	617	18.2	655	19.3	15
PC + linker	630	634	25.5	662	10.5	300
PEC	573	573/598 ³⁾	25.9	645	13.1	30
APC	653	-	-	-	-	-
PBS	632	625	4.6	660	2.1	1440
<u>Tolipothrix distorta</u>						
PC (trimer)	613	623	37.2	657	10.2	180
<u>Spirulina platensis</u>						
PC (trimer)	620	621	26.4	657	9.7	60

1) Relative change in % of maximum absorbance before reaction

2) Time required for reaching 50% of final absorption change

3) double maximum

The absorption maximum shows a monotonous red-shift with in-

the difference spectrum shifts first to the red with increasing aggregate size, but then again to the blue in PBS. However, the amplitude of the difference spectrum in PBS is much decreased as compared to smaller aggregates. Possibly, part of the β -84 chromophores are then inaccessible to the reagent, and the difference spectrum reflects only the accessible fraction. The much slower kinetics of the reaction, and the comparably high energy of the modified chromophores would be compatible with this interpretation.

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