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Section a
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Section b
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Contents

Contents of Number 1/2

Original Communications

Contents of Number 3

Original Communcations

Contents of Number 4

Original Communications

Contents of Number 5

Original Communications

Contents of Number 7/8

Original Communications

Contents of Number 9/10

Original Communications

Contents of Number 11/12

Chromophore Assignment in C-Phycocyanin from *Mastigocladus laminosus*

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Photosynthesis, Cyanobacteria, Energy Transfer, Antenna Pigments, Cystein Modification

C-phycocyanin from the cyanobacterium, *Mastigocladus laminosus*, and its subunits have been treated with *p*-chloromercuribenzenesulfonate (PCMS). A single reactive site was found on the β-subunit, and assigned to the single free cystein-β109. The concomitant spectral changes (absorption, fluorescence, circular dichroism), together with the known close proximity of cys-β109 to chromophore β82, allowed an unambiguous assignment of the three spectrally, biochemically and functionally different chromophores to specific binding sites on the two peptide chains (α84: 616–618, β82: 622–624, β153: 598–600 nm).

Introduction

C-phycocyanins (PC), the light-harvesting pigments from cyanobacteria, are composed of two subunits (α, β) bearing one and two open-chain tetrapyrrolic chromophores, respectively [1–5]. In spite of their common structures [1, 2, 6, 7], the three chromophores differ in their spectroscopic properties [8–10] and their reactivities [10–12]. These differences, which arise from the different environments of the individual chromophores in the native chromoprotein, are essential for the fine tuning of their biological functions [1–5, 8, 10, 13].

The individual environments and conformations of the three chromophores, have recently been mapped in detail by sequenation [14–19] and X-ray crystallography [20, 21]. The data indicate a considerable degree of homology around the respective chromophores in PC's from different organisms. The single chromophore on the α-subunit is attached to Cys-84, and the chromophores on the β-subunit to Cys-82 and Cys-153. The two PC's studied by high-resolution X-ray crystallography [20, 21] (from *Mastigocladus laminosus* and *Agmenellum quadruplicatum*), have furthermore almost identical chromophore and protein conformations and differ only in their aggregation state.

A correlation between the spectrally and structurally distinct chromophores has hitherto been lacking, but is very important in view of the recent progress in energy transfer studies (see *e.g.* [13]). Based on the observation of a single binding site (cys-β109) for mercurials [20, 21], we have now titrated PC and its subunits from *M. laminosus* with the thiol reagent, PCMS, which led to the unambiguous assignment of the chromophores. This situation is more clear-cut than in other biliproteins, *e.g.* phycoerythrin [22] containing more than a single free cystein available for reaction with mercurials [1–5].

Materials and Methods

M. laminosus was grown in Castenholz medium [23] in 300 l batch cultures [24] and provided to us and stored deep frozen. PC and the subunits were prepared as described previously [6]. *p*-Chloro-mercuri-benzenesulfonic acid (PCMS, Aldrich) was a gift of T. Schirmer, all other chemicals came from Merck (Darmstadt).

Absorption spectra were recorded on a model 8451 A spectrophotometer (Hewlett-Packard), fluorescence spectra on a model DMR22 fluorimeter (Zeiss, Oberkochen) and circular dichroism spectra on a model V dichrograph (Jobin-Yvon, Unterhaching).

Titrations were carried out by adding aliquots of a stock solution of PCMS (2.26 mM, in potasium phosphate buffer, 100 mM, pH 7.0) to the chromoprotein solutions (1–3 μM in the same buffer). The following extinction coefficients were used for the concentration determinations: PC trimer = 870,000 cm^2M^{-1} corresponding to 290,000 per protomer $(\alpha\beta)_1$ and 97,000 per chromophore); β-subunit = 164,000 corresponding to 82,000 per chromophore; α-subunit = 122,000. They were determined by unfolding

Reprint requests to Prof. Dr. H. Scheer.

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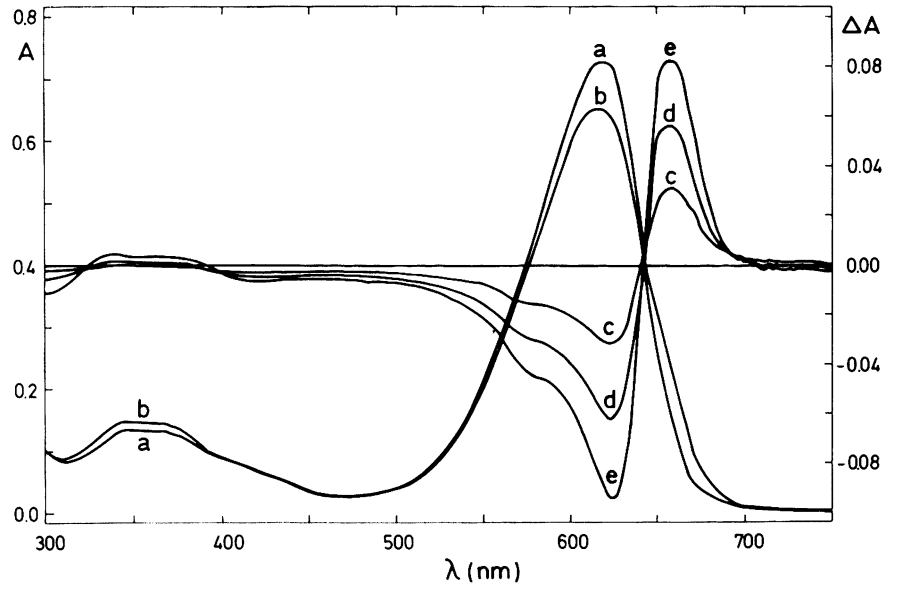
the proteins with 8 M urea, on the basis of an extinction coefficient of 15,400 per chromophore in the denatured state [11].

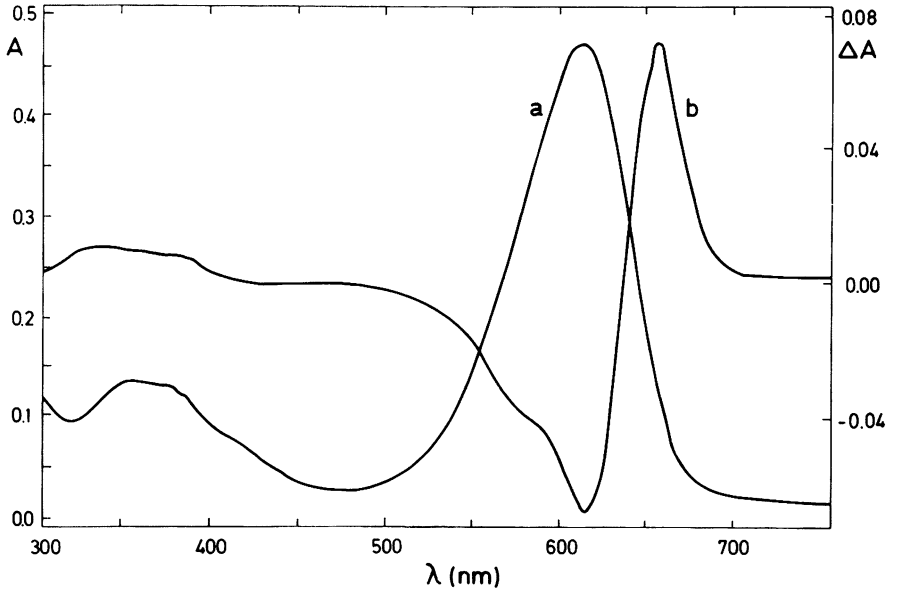
Results

Integral PC

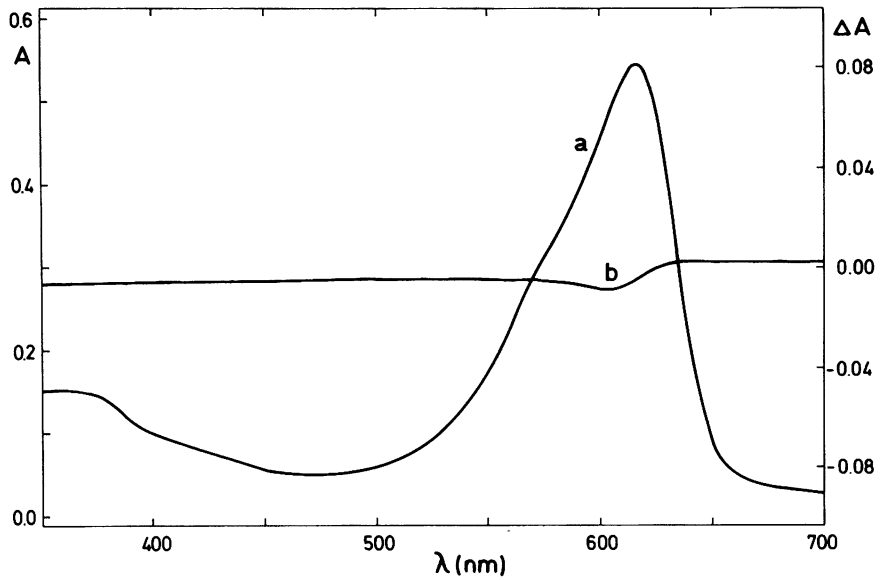
Treatment of PC trimer with a threefold molar excess of PCMS (on a monomer basis for the protein) results in a partial bleaching of the major absorption (612 nm) (Fig. 1a). It is accompanied by a minor blue-shift and the formation of a longer-wavelength shoulder. The integrated negative band (612 nm) in the difference spectrum, is about twice as large as the concomitant increase (655 nm). There is also a distinct increase in the near-uv band. Titration of PC gives a saturation of the reaction at 1 mol PCMS per mole PC (Fig. 2a). The same general features are observed in PC trimer containing linker peptides ($\lambda_{max} = 629$ nm), but here the negative peak in the difference spectrum is located at 632 nm (data not shown).



1A



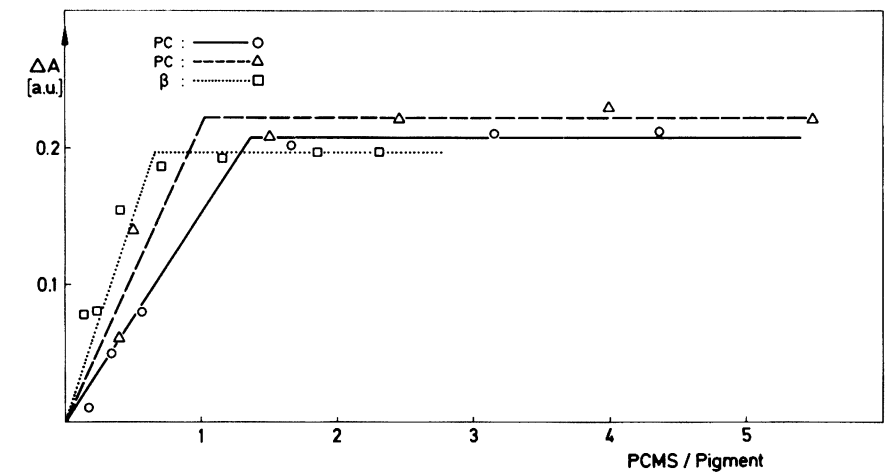
1B



1C

Fig. 1. Treatment of integral PC-trimer (A), its β-subunit (B) and its α-subunit (C) with PCMS. Absorption (left scales) and absorption difference spectra (right scales). 1A: PC (4.8 nmol, spectrum a) treated with 13.6 nmol PCMS. Difference spectra recorded after 30 (c), 60 (d) and 100 min (e), final absorption spectrum (b) 180 min after addition. 1B: Absorption spectrum (a) of the β-subunit (5.66 nmol) and final difference spectrum (b) after addition of PCMS (9.06 nmol, $t = 18$ min). 1C: Absorption spectrum (a) of the α-subunit (6.9 nmol) and final difference spectrum (b) after addition of PCMS (9.1 nmol, $t = 10$ min).

2A



2B

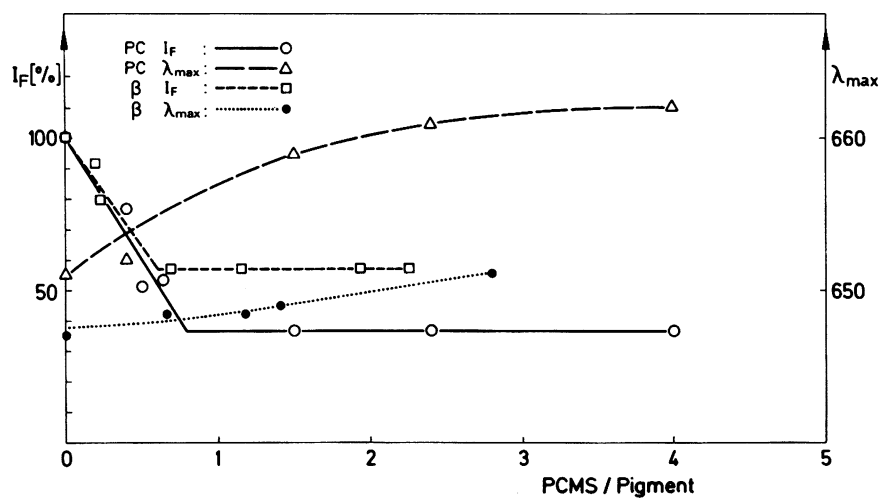


Fig. 2. Titration of integral PC and its β-subunit with PCMS. 2A: Amplitude of the absorption difference signal (maximum-to-minimum). The absorptions before addition of the reagent were 0.68 (----), 0.62 (- - -) and 0.66 (···). Labels see inset. 2B: Relative fluorescence emission intensities (I_F, left scale) and maxima (λ_{max} in nm, right scale). Labels see inset.

The fluorescence of PC trimer is reduced to a minimum of 35% of the original value and saturates at ≈ .8 mol PCMS, its wavelength is shifted by 10 nm to the red (Fig. 2b). In the CD spectrum, the most notable feature is the development of a shoulder on the red-wing of the long-wavelength band at 650 nm (data not shown, similar to the β-subunit shown in Fig. 3).

Subunits

The spectral changes of the β-subunit are very similar to the ones observed in trimeric PC. The absorption difference is again centered to the red of the absorption band. The amplitude of the difference spectrum (minimum to maximum) is even larger than PC in integral (Fig. 1b), and the reaction saturates at ≈.65 mol PCMS (Fig. 2). The fluorescence decreases by 45%, and the amplitude of the red CD maximum by 7% with a concomitant rise of a shoulder around 650 nm and an increase of the near-uv band by 17% (Fig. 3).

The α-subunit is essentially unreactive with PCMS (Fig. 1c). There is only a minor feature in the difference spectrum with an intensity of 6% as compared to the β-subunit, which probably corresponds to a minor contamination with the latter (≈5% according to SDS-PAGE). The changes in the fluorescence and the CD spectra are negligible as well (data not shown).

Discussion

Besides the three cystein residues bound to the chromophores, PC contains only a single free cystein at position 109 on the β-chain. In the native chromoprotein, this cystein is very close (≈4A) to the β82 chromophore, with an essentially protein-free hole between them, which is the single binding site for mercurials used for heavy-atom substitution [20, 21]. The distances to the other chromophores (α84, β153) both on the same monomer and on the other monomers in the trimer are considerably larger (≥22A). It is, therefore, expected that the β82 chromophore is affected rather specifically upon binding of mercurials. A differential response of individual chromophores has earlier been reported for phycoerythrin [22], but could not be explored further due to lack of structural data at that time.

The saturation behavior in solution confirms the binding of only a single molecule of PCMS both in the PC protomer and its β-subunit. This is further corroborated by the lack of reaction with the α-subunit bearing no free cystein. All spectra can be rationalized by a single chromophore absorbing around 620 nm in the β-subunit and the trimer, and around 630 nm in the linker-containing trimer, respectively, being affected by the reaction of the free cys-109 with PCMS. Two spectrally [10, 13], chemically [10, 11] and photochemically [12] distinct chromophores have been identified on the β-subunit

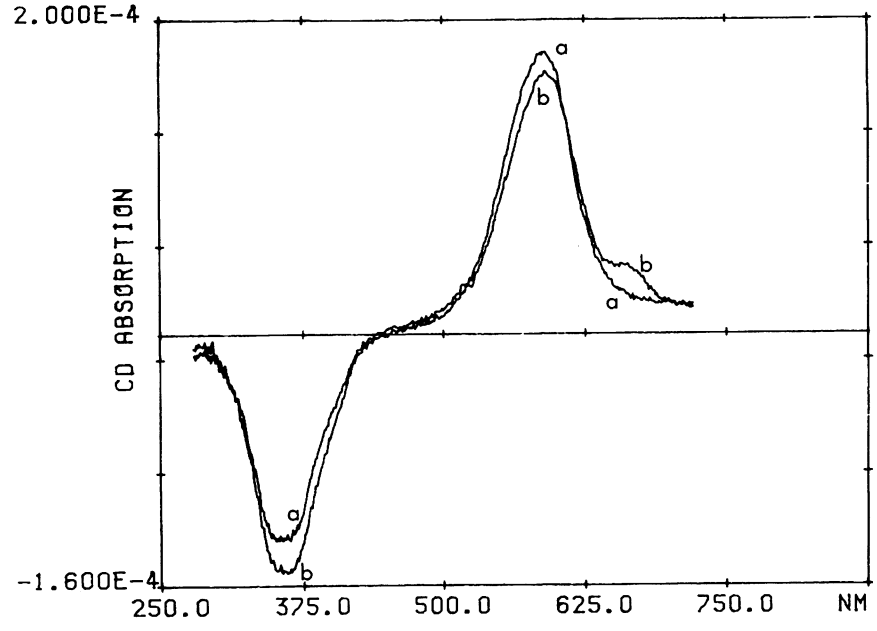


Fig. 3. Circular dichroism spectra of the β-subunit before (a) and after (b) reaction with PCMS. The spectra have been taken from the samples shown in Fig. 1B in absorption (traces a and b).

of PC from *M. laminosus*. One of them (β1) absorbs around 620 nm, is only weakly optically active and strongly fluorescent (in the absence of any acceptors), and the reversible photochemistry observed in partly denatured PC or its β-subunit resides on it. The other one (β2) absorbs below 600 nm, is strongly optically active and weakly fluorescent due to efficient energy transfer to β1 and (in integral PC) to the α-chromophore(s), and is photochemically inactive. It is clear from the data, that the former chromophore (β1) is the one close to the PCMS binding site and hence identical with β82, whereas the latter (β2) is identical with chromophore β153. If judged from the absorption changes (increase in the near-uv, decrease in the visible band), the β82 chromophore assumes a more helical conformation [11] after PCMS binding.

This assignment relates the photochemical reactivity to the chromophore, β82, which is least well defined in the X-ray structure [20, 21] and hence probably also least rigidly bound. The data obtained with the linker-containing trimer ($\lambda_{max} = 629$ nm) also indicate, that the β82 chromophore is the one shifted towards longer wavelengths (from ≈622 to ≈632 nm). These linkers, which are substantial in the spectral modulation and organisation of biliprotein aggregates, are most likely located in the inner hole of the doughnut-shaped tri- and hexamers [4, 5]. In PC, the β82 chromophore partly extends into this hole, which nicely fits with its spectral shift upon binding of linker peptides.

Treatment with the mercurial, PCMS, thus allows a straightforward chromophore assignment in PC and its aggregates. It should be useful as well with other biliproteins in view of the increased structural data available [1–5]. Interestingly, there are also large differences among the binding kinetics of PCMS to the different PC aggregates including phycobilisomes. This may be helpful in topological studies and is currently explored.

Acknowledgements

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Note added in proof: The amino acid residue numbering of the β-subunit has been used differently in the literature [14, 20]. The one used corresponds to the original sequence data [14].

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