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ZZE-Configuration of Chromophore β-153* in C-Phycocyanin from *Mastigocladus laminosus*

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Dedicated to Professor W. Nultsch on the occasion of his 60th birthday

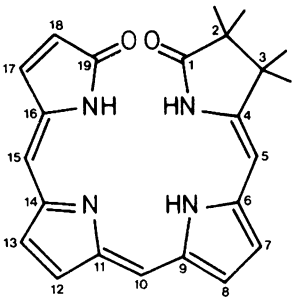
Chromophores, Isomerization, Phytochrome, Cyanobacteria

The photochemistry of C-phycocyanin has been studied after denaturation in the dark. It shows an irreversible reaction which has characteristics of a *Z,Z,E*- to *Z,Z,Z*-isomerization of dihydrobilins. Its amplitude depends on the reaction conditions, with a maximum corresponding to 15% conversion of one of the three PC chromophores. This chromophore is suggested to be β-153, for which recent X-ray data T. Schirmer, W. Bode, and R. Huber, J. Mol. Biol., submitted, show ring D being highly twisted out of the plane of the other rings. During unfolding, there is thus a probability of falling into the photochemically labile *Z,Z,E*-configuration.

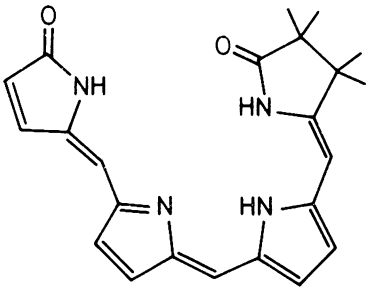
Introduction

The importance of configurational isomerization of bile pigments in photobiology has been recognized only recently. The primary reaction of phytochrome, the photosensory pigment of plants, involves the reversible light-induced *Z*–*E* isomerization of its A-dihydrobilindione chromophore at the 15, 16 double bond [5–8]. Spectrally similar reactions have been observed in partly denatured phycobiliproteins [9–12] and located in one case on a specific chromophore [13]. The isomerization of bilirubin has been recognized as an important mechanism operative in phototherapy of neonatal hyperbilirubinamea, by enhancing the solubility in water [14, 15]. *Z*–*E* isomerization(s) have also been proposed as the first step during formation of the neobiliverdins, insect bile pigments containing vinyl-group derived C-1 or C-2 bridges between pyrrolic rings [16, 17].

For bile pigments with the natural (= protoporphyrin IX derived) substitution pattern, the *Z*-configuration of double bonds bridging two pyrrolic rings (see Fig. 1) is generally thermodynamically



4Z, 10Z, 15Z



4Z, 10Z, 15E

Fig. 1. Schematic formulas for the A-dihydrobilindione chromophore of C-PC in the all-*Z* and 15*E* configuration. Substituents omitted.

Abbreviations: PC, C-phycocyanin; PE, phycoerythrin, *M*, Mastigocladus.

* We use the amino acid residue numbering of ref. [1] here, which corresponds to the original sequence data [2] but ignores homology considerations (see ref. [3, 4]).

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most stable [17–19]. The reactivity of such double bonds is dependent on their position and structural details: Whereas only the $E \rightarrow Z$ (but not the reverse) reaction is possible in biliverdin type pigments at C-4 and C-15 [20, 21], both isomers are mutually interconvertible in other cases. It has been proposed that $E \rightarrow Z$ conversion at C-10 is a normal photoreaction in biliverdins [17]. If the 10*E* isomer cannot be trapped, as it is the case in neobiliverdins [16], rapid dark reversion to the 10*Z* isomer leads to an overall radiationless deactivation [17]. In native phytochrome, the reversion is avoided by the interactions with the protein [7]. In phycocyanin (PC), on the other hand, it has tacitly been assumed that the protein stabilizes the chromophores in the all-*Z* configuration [7, 22] and that it is therefore photostable. The crystal structure of two PCs from different sources has now shown one of the chromophores, *e.g.* β-153, to be present in a conformation in which ring D is almost perpendicular to ring C [3, 4, 23]. With an angle greater than 90°, this geometry corresponds at least formally to an E-configuration of the 15, 16 double bond (see Fig. 1). This notation is only meaningful (or has only consequences for the chemical reactivity) if the 15, 16 bond is a true double-bound. This cannot be deduced from the X-ray data alone. This was the reason to check the photochemical reactivity after unfolding the protein of PC.

Materials and Methods

M. laminosus was grown in Castenholz medium [24] in 300 l batches at the Gesellschaft für Molekularbiologische Forschung, Stöckheim. PC was prepared according to previously published procedures [25]. For denaturation, the lyophilized pigment was dissolved in the dark in phosphate buffer (0.1 M, titrated to pH 1.9 with diluted hydrochloric acid) containing urea (8 M) to a final extinction of 0.3 to 0.6 at the long wavelength maximum (662 nm). Spectra were recorded without delay after dissolution and irradiation. *ZZE*-phycocyanin was prepared in denatured state (8 M urea, 0.1 M phosphate, pH = 7.0) by a modification of the method for *ZZE*-PC peptides [27]. Absorption spectra were determined with a ZWSII spectrophotometer (Biochem, Puchheim) in split beam mode with thermostated cell holders. One of the cuvettes could be irradiated in the photometer by a Lumilux 150 W light source (Volpi), equipped with a light guide (approx. 10,000 lux at the surface of the sample). Analog data were digitized (Technomar model) and then fed to a microcomputer (Apple IIe) [26].

Results and Discussion

The visible absorption spectrum of PC was determined immediately after dissolving the pigment in acidic denaturating buffer in the dark. The solution was then irradiated for 30 sec and a difference spectrum recorded. It shows a decrease in absorption at 575 nm and an increase at 673 nm (Fig. 2A). The amplitude of these signal increases with irradiation time and saturates after approx. 2 min. After saturating irradiation, the amplitude of the s-shaped difference signal is approx. 1.5% of the final absorption of this sample. If it is irradiated further for prolonged times, this fast photoreaction is followed by a slower one which gives rise to a bleaching of the chromophores. The difference spectrum of the fast reaction is very similar to the difference spectrum (min.: 575 nm, max.: 671 nm) obtained after irradiation of denatured 4*Z*, 10*Z*, 15*E*-PC (Fig. 2C), pre-

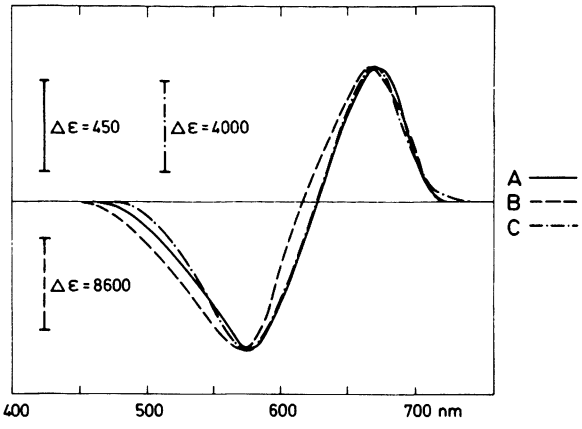

Fig. 2A. (———) Absorption difference spectrum of denatured phycocyanin (8 M urea, pH 1.9), after 2 min irradiation with white light. The reference is the unirradiated sample. The time spent after treatment of the lyophilized C-PC with the solvent was 15 min (ε_{662} = 90500/3 chromophores).
B. (– – –) Absorption difference spectrum of 4*Z*, 10*Z*, 15*E*- *minus* 4*Z*, 10*Z*, 15*Z*-bilipeptide of C-PC from *Spirulina platensis* (from ref. [27]) (ε_{661} = 90900/3 chromophores).
C. (–.–.–) Absorption difference spectrum of denatured 4*Z*, 10*Z*, 15*E*- *minus* 4*Z*, 10*Z*, 15*Z*-phycocyanin (8 M urea, pH 2.8), after 30 sec irradiation with white light (ε_{662} = 90500/3 chromophores). All spectra are normalized for equal absorption at 575 nm; see bars for absolute scale.

pared from urea-denatured PC *via* irradiation of its mercaptoethanol-adduct. The positions and relative intensities are also similar to difference spectra for photoisomerization in synthetic A-dihydrobilindione model compounds [21] and PC chromopeptides [27] with 15*E*-configuration (Fig. 2B). In all these cases, the spectral shift is much larger than that expected for the 4*E*, 10*Z*, 15*Z*-isomer, in which only the 4-double bond is uncoupled [21]. In A-dihydro-pigments like phytochrome and phycocyanin the 4*E*-isomer seems anyhow to be thermodynamically less stable than the 15*E*-isomer. Since the reaction kinetics, its pH dependence and the irreversibility are also the same, we conclude that the reaction seen in denatured, but otherwise untreated PC is also an $E \rightarrow Z$ isomerization at the 15, 16-double bond.

In pure *ZZE*-chromopeptides from PC of *S. platensis*, the difference amplitude after irradiation is approx. ⅓ of the final absorption in the *ZZZ* form [27]. If just one out of the three chromophores were to be in the *ZZE* configuration, an amplitude of approx. 10% of the original absorption would be expected.

The amplitude of denatured and acidic PC of *M. laminosus* is much smaller, however, indicating that only a small fraction of the chromophore is present in the *ZZE* configuration. The observed maximum yield of 1.5% would then correspond to a maximum of 15% of one of the three chromophores being *ZZE* after denaturation. The amplitude of the difference signal is furthermore variable (0–1.5%), with the amplitude depending on the history of the sample.

The largest effect has so far been observed in lyophilized samples. With decreasing pH it first increases up to pH 1.9 at a constant temperature of 15 °C in the sample, and decreases again at lower pH. In the same way, there is a decrease of the difference maximum with rising temperature at the optimum pH of 1.9. The origin of the increase is still unclear. The decrease may be due to a dark reaction. If the sample is kept in the dark after denaturation (pH 1.9, 6 °C), there is a slow conversion within two weeks. After 24 h, the maximum yield is reduced to about 70%, after 4 days there is 65% and after 14 days only 25% left. That means, the biggest signal could be observed, when lyophilized PC was dissolved in acidic urea buffer in the dark and the spectrum recorded at once.

The results show, that a small but distinct population of chromophores is present after denaturation in the *ZZE* configuration. According to the X-ray results [3, 4, 23] the chromophore β-153 is a likely candidate for this. The results also show, however, that only a fraction of the chromophore is present in this metastable configuration. A likely explanation would be that the native state has a geometry, which is close to an energy maximum in the denatured chromophore. During denaturation it can fall into either the *ZZE*- or *ZZZ* configuration. The different yields of the *ZZE* isomer would then reflect small variations in the energy profile of the native state and/or the denaturation path, with response to variations in temperature, pH, etc. This isomerization of β-153 in the native state contrasts with the isomerization of β-82 in partly denatured PC [13].

This result may explain several earlier observations indicating distinct differences among the chromophore β-153 and the two remaining chromophores, α-84 and β-82. It is located on a insertion of the polypeptide chain, and its absorption is shifted considerably to the blue. Whereas α-84 and β-82 absorb close to each other around 616 and 622 nm, two independent spectral analysis yielded absorption maxima below 600 nm for β-153 (598 nm in ref. [1, 28]; 594 nm in ref. [29]). The presence of spectrally distinct chromophores absorbing in these regions had originally been inferred from fluorescence polarization studies [30, 31], and has been observed in other phycobiliproteins as well. The third observation comes from NMR data of PC chromopeptides, which were distinctly different for α-84 and β-82 on one hand, and β-153 on the other hand [32, 33].

The previous rationalization for these different properties of β-153 was quite different. Non-covalent chromophore-protein interactions of an unspecified nature were postulated to account for the heterogeneity of the optical spectra [30]. The absorption spectra of bile pigments are sensitive to a variety of factors like state of protonation, conformation, charges in the vicinity, which can be controlled by the next neighbors in the native protein. A different binding site was, on the other hand, suggested as the origin for the NMR differences [33]. From a comparison with model compounds, the NMR spectrum of the chromopeptides bearing chromophore β-153 was very similar to that of a model compound bearing a thioethyl substituent at C-18 *(exo)* rather than at C-3 *(endo)*.

The results reported here suggest a different origin for the distinct properties of β-153, namely its different configuration at C-15. This tentative assignment suggested from the X-ray crystal structure, together with tests relating to the variable yields, are currently under investigation. It would also be interesting if this is true for PC from other species and for other phycobiliproteins. The occurrence of such unusual and – at least in the free chromophores – very unstable and light-sensitive structure also raises questions about its (bio)synthesis and the effect of light on it.

Acknowledgement

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