INTRODUCTION

Phycoerythrocyanin (PEC) shows, unlike other phycobiliproteins, a photoreversible photochromism reminiscent of that of the plant photoreceptor phytochrome, but with orange/green rather than red/far red action spectra. The photochemical activity is due to the photoviolobilin chromophore (λ_{\text{max}} \approx 570 \text{ nm}) located on the a-subunit. Upon irradiation with orange light, a new absorption arises at \approx 500 \text{ nm} (P506), which is converted back reversibly to the 570 nm form (P571) upon irradiation with green light. There are several lines of evidence indicating that this photochemical response involves, as in the case of phytochrome, a reversible Z/E-isomerization at the double bond between rings C and D of the linear tetrapyrrole.

Phycoerythrocyanin is commonly considered an antenna pigment, located together with phycocyanin and allophycocyanin in the phycobilisomes of certain cyanobacteria. There is presently no obvious physiological function for its photochromic responses has been characterized by absorption and absorption difference spectroscopy. Type I is the well-known absorption shift from 571 to 506 nm. Type II is a new response characterized by a line-broadening of the 570 nm absorption.

RESULTS

Separate preparations of PEC under apparently the same conditions yielded preparations of the PEC a-subunit with rather different photochemical activities. The orange/red response (measured in ΔA_{a} units) varied between 12% and 92%, and was generally on the high side. These variations are even more extreme than the reported values of 38% and 50% and are mainly related to the status of SH-groups.

The variations in photochemical activity of the a-PEC samples of different photochemistry were accompanied by a shift in the absorption maximum of the red-absorbing form (enriched after 500 nm irradiation) from 561 nm (lowest) to 566 nm (highest photochemistry). Published spectra also follow this correlation: Siebzehnrübl et al. report λ_{\text{max}} = 562 nm for a sample with ΔA_{a} = 38%, and Kullen and Björn report λ_{\text{max}} = 564 nm for a sample with ΔA_{a} = 50%. Furthermore, the low photochemistry sample showed a broader absorption and a less obvious shoulder after irradiation at 500 nm than the high photochemistry one (Figs. 1 and 2).

The difference spectra showed even more distinct changes, depending on the irradiation program. After subsequent irradiations at 500 and 570 nm, the difference spectra of high-photochemistry samples were always dominated by the well-known s-shaped type I signal (Fig. 1A). A rather different signal which has a w-shape typical for line broadening and a much smaller amplitude, was most clearly seen after subsequent irradiations at 577 and 600 nm (Fig. 1B). To distin-
guish it from the classical type I response, it shall be called type II response. For quantifying this response, we define the $\Delta \Delta A_{500}$ as for the type I response, but with the extrema at $\approx 570$ and $\approx 595$ as the reference wavelengths (see Materials and Methods).

The type II response was more pronounced in the low-photochemistry samples. Subsequent irradiations with 500 and 600 nm light resulted in a low amplitude ($\Delta \Delta A_{500} \approx 3.8\%$), somewhat distorted type I-signal ($\lambda_{\text{max}} \approx 500$ and 570 nm, Fig. 2). Subsequent irradiations at other wavelength pairs (577/600 or 500/577) resulted, in contrast, mainly in w-shaped absorption differences characteristic of the type II response (Fig. 2), with $\Delta \Delta A_{500}$ values $>10\%$. Obviously, both signals are present in all samples, but the relatively small w-shaped one is prominent only when the “classical” photochemistry of PEC is low, either by internal factors$^{17}$ or by external ones (viz. the irradiation program).

**DISCUSSION**

The photochemistry of PEC $\alpha$-subunit was discovered by G.S. Björn$^4$ during the search for cyanobacterial photochromic pigments (phycocyanins) and has since been studied in considerable detail. Intensive investigations of its photochemistry at 15,16-double bond between rings C and D, a similar isomerization has been suggested to occur during this type I photochemistry in the $\alpha$-84 phycocyanobilin chromophore of PEC.$^5,6,17$ The two distinct differences between the photochemistry of both pigments are also compatible with this interpretation: The absorption of the Z-isomer is bathochromically shifted in phytochrome and hypochromically shifted in $\alpha$-PEC, but it has been realized (albeit not understood on a molecular basis) for a long time that the bathochromic shift in phytochrome is unusual, whereas the hypochromic shift is common for 15Z-15E isomerizations in bile pigments.$^9$ The other difference is the small (apparent) reorientation of the chromophore at the 15,16-double bond between rings B and C. Although such isomers are unstable at ambient temperatures in solution,$^{18,19}$ they might be stabilized by the protein. The second possibility is a Z/E-isomerization at the methine bridge between rings C and D, e.g. the same process as in the type I reaction. However, due to a modification in the protein,$^{22}$ there may be a different interaction of the Z-isomer with the environment. Since the spectral features of biliproteins are mainly determined by such interactions,$^{20,22}$ this can lead to rather different spectra. An example for such interactions is Pfr, which as an E-isomer absorbs at longer wavelengths than the Z-isomer.$^{23}$ A third, more exotic possibility would be the formation of another form of the Z-isomer by a process reminiscent of photophysical hole-burning. The Z-E photoisomerization requires a considerable reaction volume and probably a certain amount of flexibility in the protein. The data presented here indicate that the type II photochemistry is not decreased in parallel, but rather remains unchanged or is even increased,$^{26}$ thus indicating that the rigidity of the protein may play a
lesser role. Line broadening is typical for photophysical hole-burning, a process which generally is observed at low temperatures only. However, a protein has some structural features which might allow similar processes at ambient temperatures by locking the chromophores in local minima of the conformational space.

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REFERENCES