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THE B-PHYCOERYTHRIN CHROMOPHORE

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Abstract—A study of the isolated subunits of B-phycoerythrin as well as the native biliprotein itself provides further support to the conclusion that phycoerythrobilin is the only chromophore in B-phycoerythrin.

INTRODUCTION

THE PHOTOSYNTHETIC biliprotein, B-phycoerythrin, from *Porphyridium cruentum* exhibits three absorption maxima at 565, 545, and 500 nm. Two subunits can be separated from B-phycoerythrin upon treatment with *p*-mercuribenzoate (PMB) at pH 7.[1] One of the subunits is soluble and red in color. It absorbs at 545 nm. Removal of PMB from it with mercaptoethanol causes the partial reappearance of the 565 nm band indicating its presence in the red subunit. The other subunit is insoluble and purple in color. It is characterized by an absorption maximum at about 500 nm.

Ó hEocha and Ó Carra[2] and Ó Carra *et al.*[3] suggested that in R-phycoerythrin, the chromophore absorbing at 565 and also at 545 nm is phycoerythrobilin and the chromophore absorbing at 500 nm is phycourobilin. Isolation of the red and purple subunit provides an opportunity to investigate the nature of the chromophore in each.

Recently, Chapman *et al.*[4] have made a comparative study of the chromophore in R-, B-, and C-phycoerythrin. They reached the conclusion that phycoerythrobilin is the only chromophore present in these phycoerythrins. This current investigation supports their conclusion in so far as B-phycoerythrin is concerned.

RESULTS AND DISCUSSION

Treating B-phycoerythrin at pH 7 (phosphate buffer) with PMB resulted in the rapid disappearance of its original 565-nm absorption band. The other two bands at 545 and 500 nm were only slightly affected. In contrast, with PMB at pH 9·6 (carbonate buffer), the original three absorption bands were lost and a new absorption band appeared at 510 nm along with a shoulder at 585 nm (Fig. 1). It is interesting to note that the absorption maxima of native phycoerythrin untreated with mercurial are unchanged from pH 5 to 10, indicating that the tetrapyrrole chromophores in the native protein are protected from the effect of pH change over a wide range. However, once the protein is dissociated with PMB, the chromophores respond to a change in pH.

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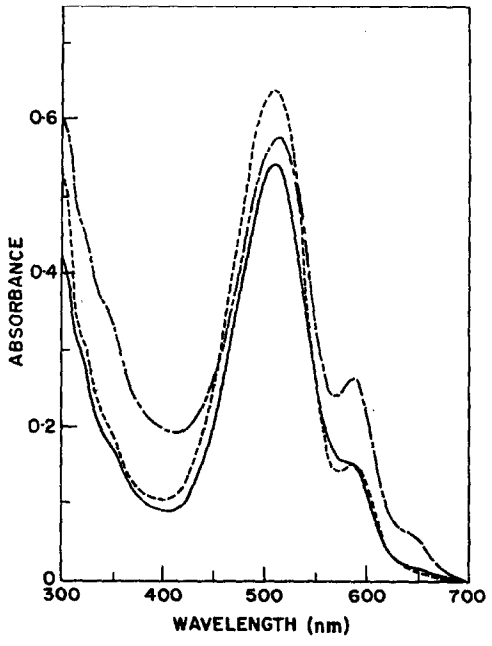

FIG. 1. ABSORPTION SPECTRA OF NATIVE PHYCOERYTHRIN AFTER 5 hr TREATMENT WITH PMB (——), THE RED SUBUNIT (----), AND THE PURPLE SUBUNIT (— · —), ALL AT pH 9·6 CARBONATE BUFFER.

When the pH of the isolated subunits is changed from 7·0 to 9·6, both exhibit the same absorption characteristics which in turn are markedly similar from that of native phycoerythrin treated with PMB at the same pH (Fig. 1). The similarity of these three spectra suggests that the chromophores responsible for the 565, 545, and 500-nm absorptions are one and the same. The chromophore at pH 9·6 has the same spectral characteristics in both the red and purple subunits, whereas at pH 7, the chromophore absorbs at different wavelengths in each subunit.

The chromophores cleaved from native phycoerythrin and the isolated subunits by methanol reflux were each dissolved in 5% hydrochloric acid–methanol (v/v) and their absorption spectra taken (Fig. 2). All three spectra clearly possess the same absorption characteristics. These spectral properties (λ_{max} 590 and 325 nm) are consistent with those of the chromophore, phycoerythrobilin or its dimethyl ester, reported by others.[4-8] This demonstrates that the same chromophore, phycoerythrobilin, is cleaved from B-phycoerythrin as well as from its isolated subunits.

The ability of the same chromophore to absorb at three different wavelengths can be attributed to the types of protein environment presented to it or to the nature of the interactions between it and the protein. There is a paucity of information concerning the exact features of these chromophore-protein interactions. It is hoped that further investigations will reveal their true character.

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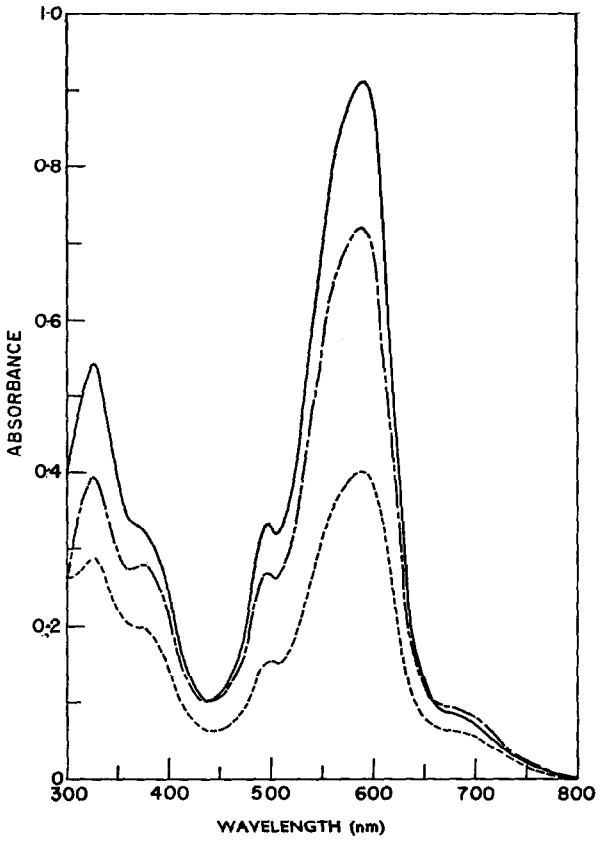

FIG. 2. ABSORPTION SPECTRA OF THE CHROMOPHORE CLEAVED FROM NATIVE PHYCOERYTHRIN (——), FROM THE RED SUBUNIT (— · —), AND FROM THE PURPLE SUBUNIT (---), ALL IN 5% HCl–METHANOL.

EXPERIMENTAL

B-Phycoerythrin from the red alga *Porphyridium cruentum* was isolated and purified as described previously.[1] Red and purple subunits were prepared by adding an excess of PMB to a solution of phycoerythrin in phosphate buffer pH 7. When dissociation was complete, the insoluble purple subunit was separated from the soluble red subunit by centrifuging at 198,000 *g* for 2 hr. The insoluble purple subunit was then washed thoroughly with buffer.

The subunits at pH 9·6 were obtained by dialyzing a solution of the red subunit and a suspension of the purple subunit, each in phosphate buffer (pH 7), against carbonate buffer (pH 9·6) at 4°. After dialysis, the suspension of the purple subunit was centrifuged at 100,000 *g* for 30 min to remove undissolved material. The supernate represented a dilute solution of the purple subunit at pH 9·6.

The chromophore from the red and purple subunits, as well as from native phycoerythrin, was isolated by the MeOH reflux cleavage method.[4–15] Both the red and purple subunits were initially freed of mercurials by adding excess mercaptoethanol and dialyzing overnight aginst two changes of H_2O. Solutions of native phycoerythrin and the red subunit were made 5% in TCA and the resulting insoluble denatured protein was

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collected by centrifugation. This step was not necessary for the insoluble purple subunit. The precipitates were then washed three times with H_2O and followed by three MeOH washings. The washed residue was then suspended in MeOH and refluxed for 16 hr with constant stirring. The refluxed mixture was then filtered through Whatman No. 1 filter paper and the filtrate diluted with an equal volume of H_2O. The resulting mixture was then extracted with 3 × 10-ml $CHCl_3$. The $CHCl_3$ extracts were combined and evaporated to dryness in a stream of N_2. The dry chromophore was kept stored at −20° and was used as shortly after preparation as feasible.

Absorption spectra were recorded using a Cary 14 spectrophotometer.