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CHANGES IN CIRCULAR DICHROISM AND ABSORPTION SPECTRUM OF PHYCOERYTHRIN CHROMOPHORES BY ORGANIC MERCURIAL

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Abstract-When B- and R-phycoerythrins and their subunits are treated with high concentrations of an organic mercurial, all chromophores including the 500-nm chromophore are converted to a similar form exhibiting two absorption bands as well as two positive CD bands at 525 and 558 mn. This ultimate chromophore state is similar to that obtained from C-phycoerythrin treated in the same way. This adds further support to the contention that the different chromophore types are due to phycoerythrobilin residues located in three different protein environments. Upon removal of mercurial with mercaptoethanol, regeneration of the original types of chromophore is only partially effected.

INTRODUCTION

THE CLASS of photosynthetic chromoproteins known as the phycoerythrins exhibit anywhere from one to three absorption maxima in the visible region.' C-phycoerythrin exhibits a single maximum at 560 nm. Double-peaked phycoerythrin, derived from single-peaked C-phycoerythrin, absorbs at 542 and 567 nm.[2] B-phycoerythrin is characterized by two closely overlapping maxima at 545 and 565 nm and a shoulder at 500 nm,[3] whereas R-phycoerythrin exhibits three distinctly separated maxima at 497, 537 and 567 nm.[4]

Previous investigations of R-phycoerythrin showed that urea, guanidine, acid or heat had no effect upon the absorption spectrum of the 500 nm chromophore.[5-7] Two other types of chromophore absorbing at wavelengths longer than 500 nm (the 540 and 565 nm chromophores) are readily affected giving rise to the same aborption maximum. This observation suggested that the 500-nm absorption was due to a different pigment.[6]

We have shown that organic mercurials can induce a two-stage change in the chromophore states of single- and double-peaked phycoerythrin both of which lack the 500-nm chromophore.[2,8] The details of the initial step leading to spectral change and dissociation of B- and R-phycoerythrins have been reported previously.[3,4,9] The purpose of this report is to demonstrate the secondary effect of mercurial upon these three-peaked phycoerythrins and their subunits. Absorption and circular dichroism (CD) changes of the 500-nm chromophore, similar to those observed with other chromophores, are revealed.

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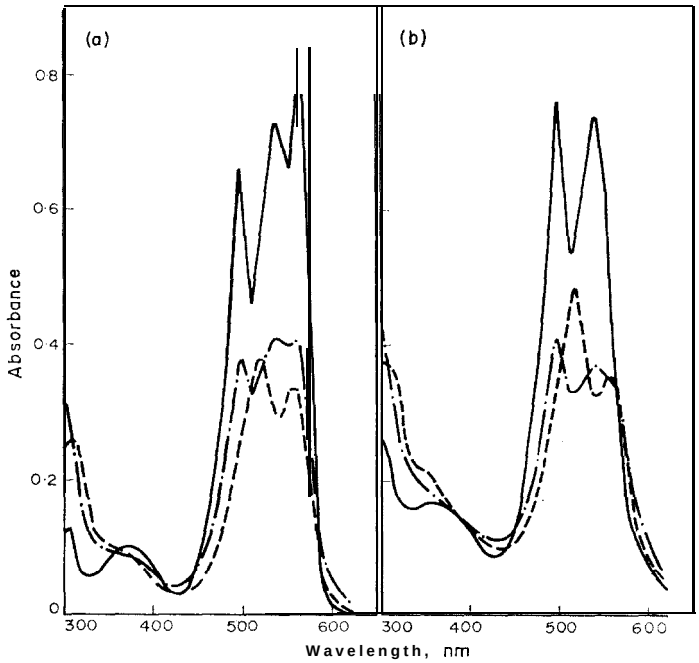

FIG. 1. ABSORPTION SPECTRAL CHANGES OF (a) R-PHYCOERYTHRIN (———) WITH 9×10^{-3} M PMPS (- - - -, 25min) AND WITH SUBSEQUENT ADDITION OF 4×10^{-2} M MERCAPTOETHANOL (——·——); (b) R-PHYCOERYTHRIN SUBUNIT (———) WITH 10^{-2} M PMPS (- - - -, 50 min) AND WITH SUBSEQUENT ADDITION OF 5×10^{-2} M MERCAPTOETHANOL (——·——).

RESULTS

The 500-nm absorption, along with other longer wavelength absorptions, of R-phycoerythrin (Fig. la), its subunit (Fig. 1 b), and B-phycoerythrin (Fig. 3a) disappeared upon treatment with between $0{\cdot}8 \times 10^{-2}$ to 1×10^{-2}M p-mercuriphenylsulfonic acid (PMPS). Two new diminished absorption bands at 525 and 558 nm resulted (Figs. la, lb, 3a). These changes were similar to those observed with the B-phycoerythrin subunit which does not possess the 500-nm chromophore (Fig. 3b). Similar changes were also observed previously with C-phycoerythrin.[2]

There was some difference in the relative intensities of the two absorption bands produced. The absorption at 525 nm was more intense than that at 558 nm in the case of R-phycoerythrin and its subunit which contain a considerable amount of the 500-nm chromophore. Nevertheless, their CD spectra (Fig. 2) underwent changes producing two positive CD bands at about 525 and 558 nm. The 525-nm CD band was less intense than the 558-nm CD band. These changes are consistent with those found with B-phycoerythrin and its subunit (Fig. 4) and with C-phycoerythrin.[8] These foregoing results demonstrate the second step of the change induced by increased concentrations of mercurial.

During the first step of protein dissociation induced by lower concentrations of mercurial, the following observations were previously made for absorption spectral changes.[3,4] The absorption band at 567 nm, although present in R-phycoerythrin, is absent in its subunit[4] (Fig. 1). The subunit of B-phycoerythrin is characterized by a single absorption band at

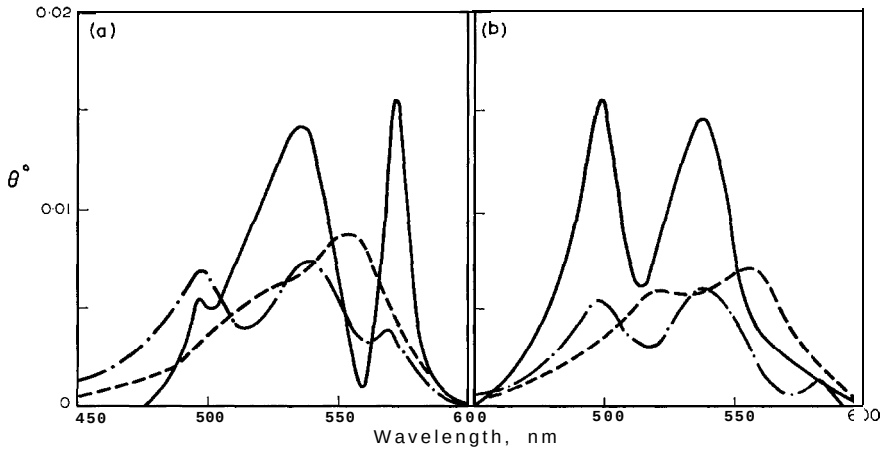

FIG. 2. CD SPECTRAL CHANGES OF (a) R-PHYCOERYTHRIN, AND (b) ITS SUBUNIT, BOTH TREATED IN THE SAME WAY AS IN FIG. 1.

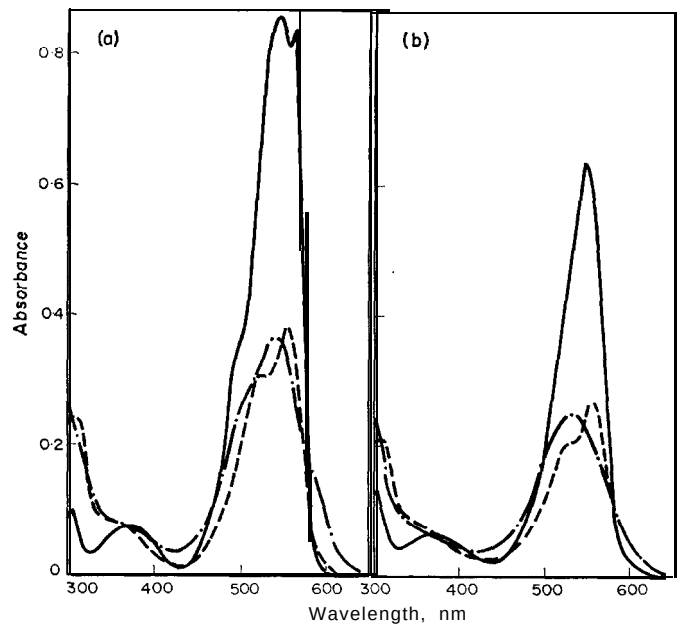

FIG. 3. ABSORPTION SPECTRAL CHANGES OF (a) B-PHYCOERYTHRIN (———) WITH 8 x 10^{-3} M PMPS (- - - -, 25 min) AND WITH SUBSEQUENT ADDITION OF 4 x 10^{-2} M MERCAPTOETHANOL (—— · ——); (b) B-PHYCOERYTHRIN SUBUNIT (———) WITH 8 × 10^{-3} M PMPS (- - - -, 15 min) AND WITH SUBSEQUENT ADDITION OF 2 X 10^{-2} M MERCAPTOETHANOL (—— · ——).

545 nm[3] (Fig. 3). Both the 565-nm and 500-nm bands are absent in this subunit. The present investigation on the CD spectra of subunits from both phycoerythrins revealed the following results. The subunit of R-phycoerythrin, which lacked the positive 572-nm CD band of R-phycoerythrin, [9] exhibited marked CD activity at about 500 nm compared to a weak

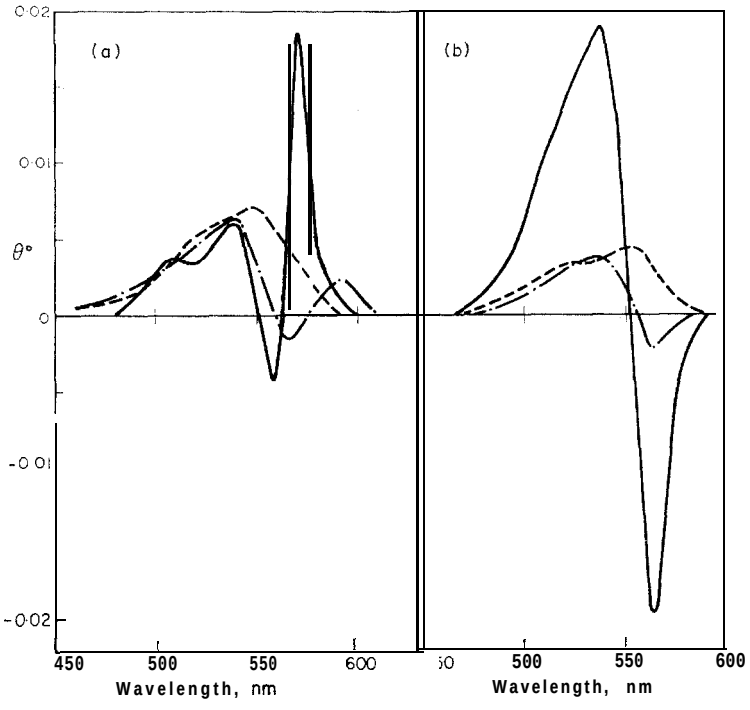

FIG. 4. CD SPECTRAL CHANGES OF (a) B-PHYCOERYTHRIN, AND (b) ITS SUBUNIT, BOTH TREATED IN THE SAME WAY AS IN FIG. 3.

CD band at 497 nm in R-phycoerythrin (Fig. 2). A double CD band of B-phycoerythrin at 572 (+) and 558 (-) nm[9] was replaced by a new double CD band of opposite sign at 564 (—) and 540 (+) nm (Fig. 4).

Despite the similarity of absorption and CD changes occurring after treatment with higher concentrations of mercurial, the manner of regeneration upon subsequent removal of mercurial with excess mercaptoethanol was different in each case particularly in its CD spectrum. Partial regeneration of the original absorption spectra was observed with R-phycoerythrin and its subunit (Fig. 1). R-phycoerythrin showed some enhancement of the positive 500-nm CD band upon regeneration, but the regeneration of the positive 572-nm CD band was less efficient (Fig. 2a). Its subunit exhibited the appearance of a weak positive CD band which had shifted to about 584 nm (Fig. 2b). After partial regeneration, the absorption spectrum of B-phycoerythrin gave rise to a new shoulder at about 590 nm instead of the 565-nm band (Fig. 3a). Its subunit regenerated no shoulder at 590 nm (Fig. 3b). B-phycoerythrin did not clearly regenerate the positive 500-nm CD band but did give rise to a double CD band, shifted to 592 (+) and 566 (—) nm (Fig. 4a). Only limited regeneration of the original double CD band was observed with its subunit (Fig. 4b).

DISCUSSION

Higher concentrations of mercurial profoundly alter the state of chromophores bound to the protein. The structural integrity of the different types of chromophore-protein interactions are lost, ultimately resulting in a form whose spectral characteristics are

similar. This observation provides further support to the suggestion that the different types of chromophore, including the 500-nm chromophore present in triple-peaked phycoerythrins, are due to the same prosthetic group, phycoerythrobilin,[10] in specific interactions with the apoprotein.[11,12] This modification of the protein-chromophore interactions is specific for mercurial and differs from the effect of protein denaturation by other reagents. The unfolding of the protein structure by urea or guanidine does not materially affect the unique state of the 500-nm chromophore.[5–7]

Phycoerythrins contain sulfhydryl groups.[1,13] Although it is well known that mercurials react with sulfhydryl groups, binding of excess organic mercurials at a number of sites other than sulfhydryl groups has been shown to occur with other proteins.[14] The chemical nature of these sites are not known at present. The stoichiometry of these phycoerythrins with mercurials remains for further investigation.

The relative intensities of the two absorption bands induced by higher concentrations of mercurial from the 500-nm chromophore differ from those for the 540 and 565-nm chromophores. Apparently there are still subtle differences in conformation reflecting the origin of the chromophores even in this state. This seemingly insignificant difference becomes pronounced after removal of mercurial. The protein and chromophore conformation responsible for the original 500-nm chromophore is partially regenerated. The regeneration of the 565-nm chromophore is most inefficient and in some cases somewhat modified with a further shift to longer wavelengths. It was previously shown that this 565-nm chromophore is most sensitive to the action of mercurial and disappears even when treated with lower concentrations of mercurial.[2–5,9,13]

EXPERIMENTAL

B- and R-phycoerythrins were prepared from ***Porphyridium cruentum***[3] and ***Ceramium rubrum***,[4] respectively. The soluble subunit was obtained from each phycoerythrin by treatment with excess *p*-mercuribenzoate.[3,4] After completion of dissociation, the subunit was separated from insolubles by centrifugation and subsequently purified by Sephadex G-100 filtration.

A Cary 60 recording spectropolarimeter equipped with a 6001 CD accessory was employed for the measurement of CD spectra. The absorption spectra ware recorded using a Cary 14 spectrophotometer. Cells with a path length of 10 mm were used in all measurements. The solvent used was 0·1 M phosphate buffer, pH 7·0.

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