

LETTER TO THE EDITOR

Reply to Tamilarasan and McMillin

Dear Sir:

Recently, Tamilarasan and McMillin (1986) discussed the changes in the near-UV absorption spectrum of plastocyanin (PC) observed upon reduction. They reported the UV absorbance of both a Hg(II) derivative and of an apoPC prepared by dialysis vs. 0.01 M CN^{-1} at 4°C in 0.025 M Tris-HCl (pH 8.05). Their Hg(II) derivative exhibited a near-UV absorption spectrum almost identical with our spectrum of PC treated with 3 eq of mercuric acetate (Draheim et al., 1986) by the method of Scawen et al. (1975). This indicates that the derivative we previously thought to be apoPC was most likely a Hg derivative.

We have now prepared apoPC by the method of Tamilarasan and McMillin and carefully measured its spectrum. The near-UV absorption spectrum we measure is similar to theirs. We agree that it is also quite similar to that of oxidized plastocyanin. We do not dispute that part of the difference between oxidized and reduced PC can be attributed to charge transfer transitions. However, the apoPC prepared by their method appears to be denatured based on its far-UV circular dichroic (CD) spectrum. We have subsequently prepared an apoPC derivative with a far-UV CD spectrum more similar to that of oxidized or reduced PC. This apoPC has a near-UV absorption spectrum different from that of oxidized PC. This will be the topic of a future publication.

We still insist that a careful interpretation of UV absorbance

and CD data for various species of PC under different pH conditions indicates the presence of protein conformational changes. Most of the arguments of our previous paper are not affected by the assignment of the Hg(II)PC spectrum.

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REFERENCES

- Draheim, J. E., G. P. Anderson, J. W. Duane, and E. L. Gross. 1986. Plastocyanin conformation. An analysis of its near ultraviolet and circular dichroism spectra. *Biophys. J.* 49:891-900.
- Scawen, M. D., E. J. Hewitt, and D. M. James. 1975. Preparation, crystallization and properties of *cucurbita pepo* plastocyanin and ferredoxin. *Photochemistry*. 14:1225-1233.
- Tamilarasan, R., and D. R. McMillin. 1986. Ultraviolet absorbance increase on reduction of plastocyanin: conformational change or a new chromophore? *Biophys. J.* 50.
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