LETTER TO THE EDITOR

Ultraviolet Absorbance Increase on Reduction of Plastocyanin: Conformational Change or a New Chromophore?

Dear Sir:

Recently Draheim et al. (1986) discussed the near-UV absorption and circular dichroic spectra of plastocyanin, a protein with a single type 1 or "blue" copper binding site. They reported that the UV absorbance of the protein varied with conditions; in particular, it was significantly increased in the reduced form of the protein ($Cu^{I}Pl$). Since they found that apoplastocyanin (apoPl) had a similar absorbance, they concluded that the absorbance changes were not directly connected with the metal center. Instead, they proposed that conformational changes were responsible and that these changes were reflected in the tyrosine absorbance. In an independent study we investigated the near-UV absorption spectrum of $Cu^{I}Pl$ as well as those of apoplastocyanin and several derivatives of plastocyanin with d^{10} metal ions present in the metal-binding site (Tamilarasan and McMillin, 1986). In contrast to Draheim et al., we found that the absorptivity of the tyrosine residues was hardly affected by the nature of the metal ion in the type 1 binding site. In addition, we determined that the near UV absorbance of $Cu^{I}Pl$ was enhanced by comparison with apoPl as well as $Cu^{II}Pl$, and we attributed the absorbance increase to copper-dependent transitions superimposed on the tyrosine absorbance. As shown below, our data can be reconciled with that of Draheim et al. when it is realized that their method of copper removal actually yields the mercury (II) derivative of plastocyanin rather than the apoprotein.

Draheim et al. (1986) report "using the method of Scawen et al. (1975)" to prepare apoPl. To remove copper, Scawen et al. combined plastocyanin with 3 eq of mercuric acetate in 0.1 M Tris-HCl buffer at pH 7.6. The protein, less copper, was separated by gel filtration on a Sephadex G25 column. Although Scawen et al. (1975) noted that the treated protein had lost copper, they did not explicitly label the protein as apoPl. They did show that copper could be reincorporated into the protein, but the copper addition was done in the presence of glutathione, which can act as a scavenger of Hg(II). We treated a sample of $Cu^{II}Pl$ with mercuric acetate and obtained modified protein that gave the absorption spectrum depicted in Fig. 1, in excellent agreement with the one reported by Draheim et al. (1986). However, the product was not apoPl because Cu(II) did not combine with the protein in the absence of glutathione. Furthermore, the procedure of Ellman (1959) showed that the sample had no free thiol group. When we analyzed for mercury by the method of Yamamura (1960) and for total protein by the biuret method (Goa, 1953), we found that the protein contained 1.0 ± 0.1 eq of Hg(II). The metal stoichiometry and the thiol titration data can be understood if mercury is bound in the type 1 binding site. In line with this conclusion, previous x-ray results have shown that a similar treatment results in the replacement of copper by mercury (Colman et al., 1978; Church et al., 1986).

To prepare an authentic sample of apoPl, reduced plastocyanin was dialyzed against 0.01 M CN^- at 4°C in 0.025 M Tris-HCl buffer (pH 8.05) as previously described (Tamilarasan and McMillin, 1986). By incubating apoPl with 1.25 eq of Hg(II) for 2 h in the Tris-HCl buffer and then dialyzing against the same buffer, a second preparation of $Hg^{II}Pl$ was effected. The absorption spectra of the two preparations of $Hg^{II}Pl$ were identical, and the circular dichroic spectrum of our $Hg^{II}Pl$ sample agreed with that reported for apoPl by Draheim et al. (1986).

The UV absorption spectra for $Cu^{I}Pl$ and apoPl are also included in Fig. 1. By comparison with an apoPl reference, both the Cu(I) and the Hg(II) derivatives exhibit enhanced absorbance in the near UV. Difference spectra reveal only positive bands at 247 nm ($\Delta\epsilon$ = 9,800 M^{-1} cm^{-1}) and at 280 nm ($\Delta\epsilon$ = 2,100 M^{-1} cm^{-1}) for $Hg^{II}Pl$ and at 275 nm ($\Delta\epsilon$ = 3,300 M^{-1} cm^{-1}) and 310 nm ($\Delta\epsilon$ = 700 M^{-1} cm^{-1}) for $Cu^{I}Pl$ (Tamilarasan and McMillin, 1986). For the Hg(II) derivative the bands can readily be assigned (Tamilarasan and McMillin, 1986) as S(Cys) → Hg(II) charge-transfer absorptions analogous to transitions found in the spectra of mercury thioneins (Vašák et al., 1981). Several possible assignments can be offered for the bands in $Cu^{I}Pl$ including 3d → 4s Rydberg transitions, Cu(I) → $^{*}\pi$(His) charge–transfer transitions and charge-transfer to solvent transitions (Tamilarasan and McMillin, 1986). A mixed orbital parentage is also possible because all of these transitions involve the radial extension of electron density from the 3d shell.

In summation, the near-UV absorbance spectra of apoPl and $Cu^{II}Pl$ are very similar, while the reduced protein, $Cu^{I}Pl$, exhibits increased absorbance. We have previously argued that the increase is due to the onset of electronic transitions involving the copper center and that any changes in tyrosine absorbance due to

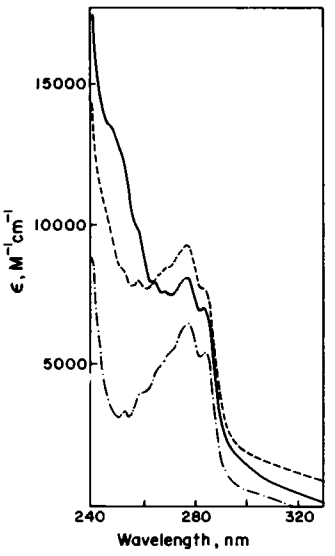

FIGURE 1 Near-UV absorption spectra of plastocyanin derivatives in 0.1 M phosphate buffer (pH 7.0) at 20°C. ApoPl (—·—); $Cu^{I}Pl$ (---); $Hg^{II}Pl$ (——).

conformational effects must be small (Tamilarasan and McMillin, 1986).

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