Electronic Spectroscopy of Gold(I) *Pseudomonas aeruginosa* Azurin Derivatives

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Introduction

The trigonal (His_2Cys) coordination of blue copper sites in proteins[1] favors Cu(I) over Cu(II), as reflected in the relatively high $Cu^{II/I}$ reduction potentials;[2] and the rigid polypeptide environment minimizes $Cu^{II/I}$ nuclear reorganization, thereby facilitating long-range electron-transfer reactions with donor and acceptor molecules.[3] Although blue Cu(II) sites exhibit rich spectroscopic and magnetic properties,[4] the corresponding Cu(I) proteins do not; indeed, the methods that can be employed to investigate d^{10} metal sites are very limited.[5] Because recent work has shown that Au(I) has geometry-sensitive d−p absorptions and emissions,[6,7] we are using this $5d^{10}$ ion to probe ligand interactions in the Cu(I) sites of proteins. Here we report the electronic spectroscopy of Au(I)-substituted wild-type (WT) *Pseudomonas aeruginosa* azurin[8,9] as well as the Au(I) derivative of a mutant in which the methionine at position 121 has been replaced with glycine (Met121Gly).[10−12]

Experimental Section

$ClAu(S(CH_2CH_2OH)_2)$ (1). $S(CH_2CH_2OH)_2$ (two equivalents) was added to a stirring solution of 1 g of $HAuCl_3$ in 50 mL of water. The resulting clear solution was reduced to a viscous oil, and **1** was isolated as a white solid by recrystallization from ethanol/ether. The solid is air sensitive (and will degrade over a period of 24 h) but can be stored in a cold ether suspension for several weeks.

Preparation and Purification of Azurins. Met121Gly *P. aeruginosa* azurin was made by oligonucleotide-directed mutagenesis using a synthetic azurin gene.[10] Both WT and mutant apo proteins were expressed heterologously in *Eschericia coli* (as described previously)[13] and purified under basic conditions (10 mM DEA-HCl; pH 8.8) by anion-exchange FPLC using a NaCl gradient. Apo protein concentrations were estimated using $\epsilon_{280} = 10^5\ M^{-1}\ cm^{-1}$. Cu(II) WT azurin was made by adding a slight excess of aqueous $CuSO_4$ to a solution of the apo protein. The resulting blue solution of the Cu(II) protein was treated with EDTA prior to FPLC purification. Cu(I) WT azurin was made by dithionite reduction of a concentrated solution of the oxidized protein. Excess dithionite and other small-molecule contaminants were removed by gel filtration. Au(I) WT and Met121Gly azurins were made by adding a 50-fold excess of **1** in 5 mL of 10 mM DEA-HCl (pH 8.8) to 5 mL of a 10 mM DEA-HCl (pH 8.8; 10 mM NaCl) solution containing approximately 10 mg of purified apo azurin. After incubation for two days, the mixture was washed and concentrated repeatedly with 10 mM DEA-HCl (pH 8.8) to remove excess **1** as well as any surface-bound Au(I). Cu(II) WT and Au(I) azurins were purified to homogeneity by the same procedure employed for the apo proteins.

Spectroscopy. Absorption spectra were recorded on a modified Cary 14 spectrophotometer.[14] Emission spectra were measured using a home-built instrument.[14]

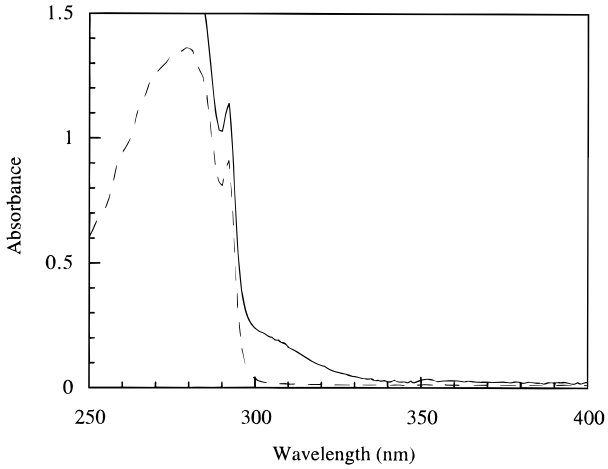

Figure 1. Electronic absorption spectra of Au(I) WT (—) and apo WT (− −) azurin in 10 mM DEA-HCl (pH 8.8, 10 mM NaCl).

Results and Discussion

The electronic absorption spectrum of Au(I) WT azurin exhibits intense absorption below 270 nm and a shoulder at 308 nm that is not present in the spectrum of the apo WT protein (Figure 1). The position and intensity ($\epsilon = \sim 10^3\ M^{-1}\ cm^{-1}$) of the shoulder suggest that the transition in question is $^1A_1 \rightarrow E'(^3E'')$[6] in a trigonal Au^IN_2S (N_2 = His46, His117; S = Cys112) complex. Occupation of Au(I) in the azurin active site[15] was confirmed by the inability to generate the characteristic blue color of type 1 azurins[4] upon addition of Cu(II) to solutions containing Au(I)-reconstituted WT azurin.

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(15) It is likely that Au(I) binds strongly to the Cys112 sulfur in the active site. Attempts to reconstitute Cys112Asp *P. aeruginosa* azurin (Mizoguchi, T. J.; Di Bilio, A. J.; Gray, H. B.; Richards, J. H. *J. Am. Chem. Soc.* **1992**, *114*, 10076−10078) with Au(I) failed. Samples of Au(I)-treated Cys112Asp azurin exhibited an absorption spectrum indistinguishable from that of the apo protein; and these samples could be reconstituted with Cu(II).

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The emission properties of Au(I) WT azurin accord well with the proposed coordination geometry. The Au(I) protein emits in the red region ($\lambda_{max} = \sim 580$ nm),[16] whereas apo, Cu(II), and Cu(I) derivatives do not emit at wavelengths > 450 nm (Figure 2). Our finding that the emission band is extremely broad (fwhm of 4300 cm^{-1}) indicates that the E′(^{3}E″) Au(I)-N_2S unit is severely distorted, as expected.[6] The emission lifetime of 30 μs at 77 K corresponds well with the ~20-μs lifetimes observed for trigonal Au^IP_3 complexes.[6]

The absorption and emission bands in the spectra of Au(I) Met121Gly azurin are blue-shifted from corresponding features in the Au(I) WT protein; also, there is a narrowing of the Au(I) Met121Gly emission band (Figure 2B). Au(I) Met121Gly azurin emits at 520 nm with a FWHM of 2900 cm^{-1}; the excitation spectrum has a band at 285 nm attributable to $^1A_1 \rightarrow$ E′(^{3}E″), a transition that is obscured in absorption. The cavity created by the smaller Gly residue allows a water molecule to enter the site[12] and occupy an axial position in the Au(I) coordination sphere. An axial water ligand would destabilize the E′(^{3}E″) excited state, owing to repulsive interactions of the oxygen donor electrons with the E′ $6p_z$ electron; the presence of this ligand also could explain the reduction in the excited-state distortion that is indicated by the narrowing of the emission band.

We have found that Au(I) can be incorporated into the active site of azurin and that the Au(I) center can be studied by electronic spectroscopy. Furthermore, we have shown that Au(I) is a useful probe for the spectroscopically silent Cu(I) ion; Au(I) substitutions could, in principle, be extended to examine the coordination sites of Cu(I) in other proteins.[17]

(16) Notably, metal-centered (p → d) emission has not been detected from mononuclear Au(I) in any centrosymmetric (*e.g.*, linear $Au(I)L_2$) coordination geometry; however, work from two laboratories (ours and Fackler's)[6,7] has shown that the E′(^{3}E″) state of trigonal planar Au(I) is strongly emissive.

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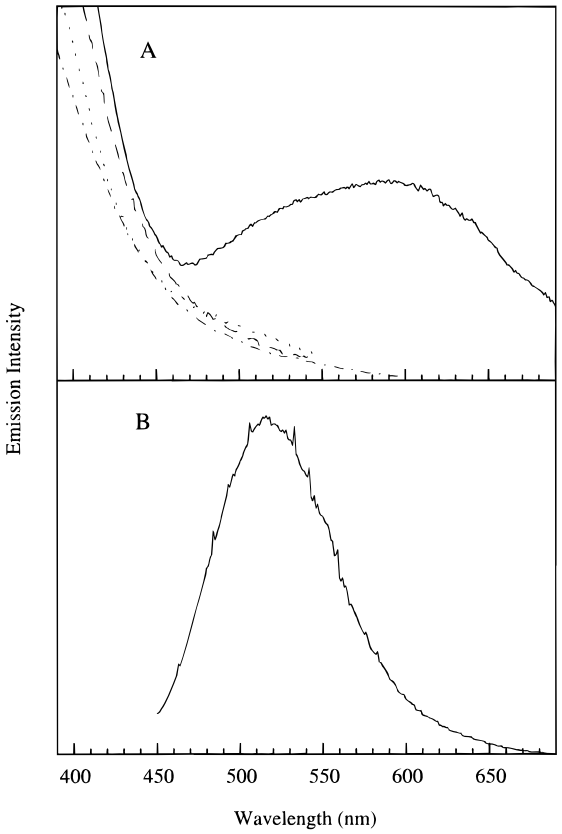

Figure 2. Emission spectra (glycerol/H_2O glass, 77 K): (A) Au(I) WT (—), apo WT (− −), Cu(II) WT (···), and Cu(I) WT (−·−) azurin ($\lambda_{exc} = 366$ nm); (B) Au(I) Met121Gly azurin ($\lambda_{exc} = 330$ nm).

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