Metal Binding to *Pseudomonas aeruginosa* Azurin: a Kinetic Investigation

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The interaction between azurin from *Pseudomonas aeruginosa* and Ag(I), Cu(II), Hg(II), was investigated as a function of protein state, i.e. apo-, reduced and oxidised azurin. Two different metal binding sites, characterized by two different spectroscopic absorbancies, were detected: one is accessible to Ag(I) and Cu(II) but not to Hg(II); the other one binds Ag(I) and Hg(II) but not copper. When added in stoichiometric amount, Ag(I) shows high affinity for the redox center of apo-azurin, to which it probably binds by the -SH group of Cys112; it can displace Cu(I) from reducedazurin, while it does not bind to the redox center of oxidizedazurin. Kinetic experiments show that Ag(I) binding to the reducedform is four times faster than binding to the apo-form. This result suggests that metal binding requires a conformational rearrangement of the active site of the azurin.

Interaction of Ag(I) or Hg(II) ions to the second metal binding site, induces typical changes of UV spectrum and quenching of fluorescence emission.