CAPSULES notes and applications from BASi

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April 2000

Substitution of Glutathione by Glutamate as the Axial Ligand in the Copper Site of Pseudomonas aeruginosa Azurin

Purpose

Although the copper active sites of blue copper electron transfer proteins are all similar, the redox potentials of these proteins vary over a range of several hundred millivolts. Since there are differences in the interactions between the copper center and the axial ligands, there has been considerable interest in elucidating the effect of varying the nature of the axial ligand on the redox potential. In this study, the glutathione-121 axial ligand in *Pseudomonas aeruginosa* (*Pa*) azurin was changed to glutamate (i.e., the axial Cu-S interaction was substituted by a Cu-O interaction), and the mutant protein was characterized at different pH values.

Reference

X-Ray Structural Determination and Characterization of the Pseudonomas aeruginosa Azurin Mutant Met121Glu, B.G. Karlsson, L.C. Tsai, H. Nar, J. Sanders-Loehr, N. Bonander, V. Langer, and L. Sjölin, Biochemistry 36 (1997) 4089-4095.

Method

Redox potentials were measured using spectroelectrochemistry. A constant potential was applied to the spectroelectrochemical cell, and, once equilibrium had been attained, the spectrum was recorded. The concentrations of the oxidized and reduced species were extracted from the spectrum, and the applied potential was plotted against the log of the ratio of the concentrations. The redox potential is the intercept of this plot (see Capsule 349 for more details).

Results

A structural motif that is common to the copper site of all blue copper proteins is the trigonal arrangement of two histidines (H46 and H117) and one cysteine (C112) (1). These provide three donors (2 N and 1S) that lie in the same (equatorial) plane, and interact strongly with the copper center. There is also axial coordination, but the number or donors, and their degree of interaction varies. In azurin, there are two weakly bound axial ligands (121M and G48) (Cu-S and Cu-O distances = 3.1 Å), and the copper center lies close to the equatorial plane (F1) (2). The copper

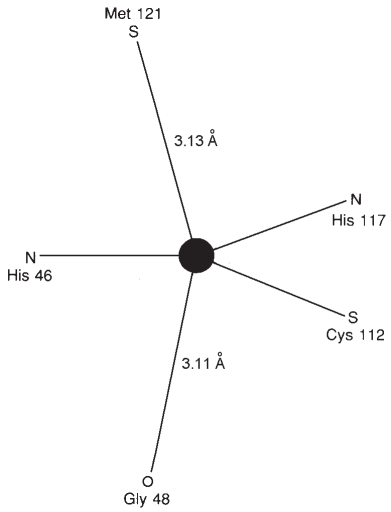

Figure 1. *Molecular structure of the active site in azurin (2).*

coordination geometry can therefore be described as a distorted trigonal bipyramid. In plastocyanin, there is only one weak axial ligand (121M) with a Cu-S distance of about 2.9 Å, and the copper center lies about 0.3 Å above the equatorial plane (i.e., the coordination geometry is a distorted tetrahdron). The coordination geometries in pseudoazurin and cucumber basic blue protein are also distorted tetrahedra (***F2***) (3), but the interaction with the axial methionine residue is stronger (Cu-S = 2.6 - 2.7 Å). In stellacyanin, the geometry is also a distorted tetrahedron, but the axial ligand is a glycine carbonyl oxygen (***F3***).

These small variations in the structure of the copper site give rise to changes in the spectroscopic properties of these proteins (3, 4). The UV/visible spectrum of azurin shows an intense transition at about 600 nm, with a weaker one at about 450 nm

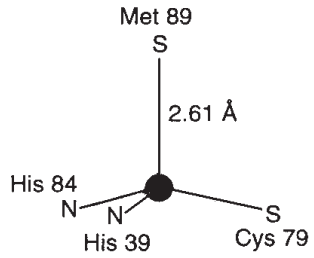

Figure 2. *Molecular structure of the active site in cucumber basic blue protein (3).*

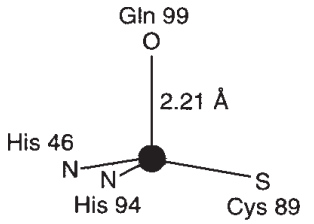

Figure 3. *Molecular structure of the active site in stellacyanin (3).*

(these have been attributed to Cys-S ® Cu charge transfer), and the epr spectrum shows axial symmetry. The charge transfer transitions are also present in the spectra of other blue copper proteins, but the relative intensity of the transition at about 450 nm increases with increasing interaction with the axial ligand. The symmetry of the epr spectrum changes from axial to rhombic as the Cu-S(axial) distance decreases.

The spectroscopic properties of the M121E mutant vary with pH. At pH 4, the UV/visible and epr spectra are similar to those of stellacyanin and pseudoazurin, which suggests axial coordination that is still weak, but stronger than in the wild type azurin. However, at pH 8, the spectra are significantly different from those of any wild type blue copper proteins (e.g., the dominant transition in the UV/visible spectrum is at 416 nm), which suggests a significant change in the coordination at the copper center.

It was shown by X-ray crystallography and EXAFS (5) that the change in the spectroscopic features with pH was due to protonation/deprotonation of the carboxylate group on the glutamate side-chain (pKa = 5.0). At pH 4, the carboxylate group is protonated, and the interaction between the copper center and this axial ligand is relatively weak (Cu-O distance = 2.21 Å). Hence, the spectroscopic properties are similar to those of other blue copper proteins, since there are three strong equatorial interactions, and one weak axial interaction. However, at pH 8, the carboxylate group is deprotonated, and the axial interaction is much stronger (Cu-O distance = 1.9 Å from EXAFS studies (5)). Therefore, there are now four strong interactions, which gives rise to spectroscopic properties different from those of the wild type blue copper proteins.

Another characteristic feature of blue copper proteins are their redox potentials (ca. +200 - +700 mV vs. NHE), which are considerably more positive than the redox potentials typically reported for copper coordination complexes. This has been attributed to the coordination of sulfur ligands, and the pseudo-tetrahedral geometry imposed on the copper center by the protein, which is more favorable for copper(I) than copper(II). More recently, the high redox potentials have been attributed to the poor electron donation from the weakly coordinated axial ligand, which destabilizes the higher oxidation state (6). The variation of the redox potential of the M121E mutant with pH (+370 mV at pH 4, +220 mV at pH 7, and +184 mV at pH 8) is consistent with the copper(II) oxidation state being stabilized by the increased electron donation from the axial ligand (for comparison, the redox potential of wild type azurin changes from +350 mV at pH 4.5 to +290 mV at pH 8.5 (7)).

References:

1. *Abbreviations: C=cysteine, E=glutamate, G=glycine, H=histidine, M=methionine*
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