Crystal Structure of a Novel Red Copper Protein from *Nitrosomonas europaea*

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Introduction

Nitrosocyanin (NC) is a unique 112 residue mononuclear copper protein found in *Nitrosomonas europaea*,[1] a chemoautotrophic bacterium that derives energy from the oxidation of ammonia to nitrite and plays a key role in the nitrification branch of the global nitrogen cycle. NC exhibits some sequence homology to blue copper proteins, such as plastocyanin and the electron transfer domain of nitrous oxide reductase (N_2OR), but its spectroscopic and electrochemical properties are drastically different.[1] NC is brilliant red in color with an absorption band at 390 nm (ε = 4400 M^{-1} cm^{-1}). Its redox potential of +85 mV is well outside the range of +184-+680 mV observed for blue copper.[2] In addition, its EPR spectrum is more similar to that of normal copper. Since NC has all of the properties traditionally associated with blue copper and its optical spectrum and sequence are not consistent with normal copper, its red copper site likely represents a new type of mononuclear copper center. To determine the molecular details of this novel red copper center and to gain insight into its biological function, we have determined the crystal structure of NC.

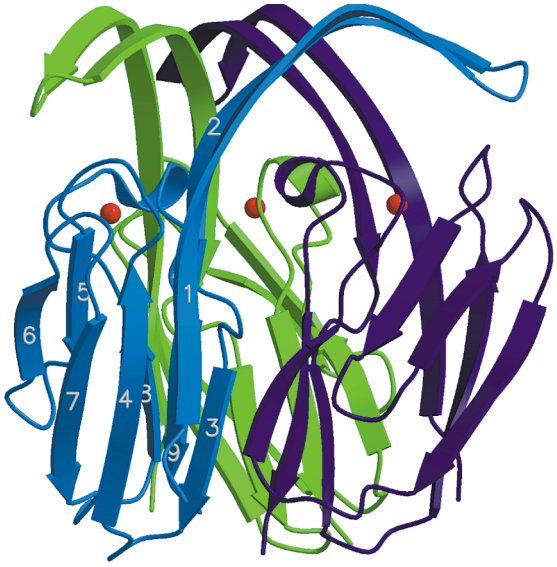

FIG. 1. The NC trimer.

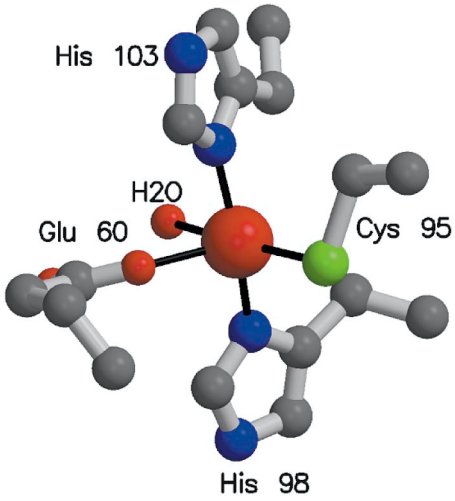

FIG. 2. The NC red copper center.

Methods and Materials

Nitrosocyanin was purified and crystallized as described elsewhere.[3] Data for Cu multiple wavelength anomalous dispersion (MAD) phasing to 2.1 Å resolution were collected at DND-CAT at four wavelengths using the reverse beam technique. The positions of six copper ions were determined using the program SOLVE.[4] An initial electron density map calculated to 3 Å resolution revealed continuous density corresponding to multiple β strands and indicated that the six copper ions are arranged in two protein trimers in the asymmetric unit. Iterative cycles of simulated annealing and individual B-value refinement with CNS[5] followed by model rebuilding with O[6] were performed using the free R-value to monitor the refinement progress. After completion of the 2.1 Å resolution structure, a 1.65 Å resolution data set was collected on a different crystal form, and the structure was refined to 1.65 Å resolution.

Results and Discussion

Three NC monomers form a cylindrical trimer (Fig. 1). Each monomer comprises a flattened, eight-stranded β-barrel (β1, β3-β9) and an unusual β-hairpin structure (C-terminus of β1 and β2, residues 11-32) that extends from, but is not part of, the β-barrel structure. Each hairpin caps the copper site of an adjacent monomer, with the copper ion ~16 Å from the top surface of the pinwheel. The hairpin forms a hydrophobic cavity, ~30 $Å^2$ in volume. The β-barrel of the NC monomer exhibits a cupredoxin fold characteristic of single domain blue copper proteins involved in intermolecular electron transfer.[7,8] The trimeric structure observed in NC is not conserved in any of these small cupredoxins, however. NC is thus the first oligomeric single domain cupredoxin. NC also resembles the cupredoxin domains of numerous multicopper enzymes.[8]

Each monomer in the NC trimer houses one mononuclear copper site, and each pair of copper ions is separated by ~23 Å. The copper ion is coordinated by the sulfur of Cys 95, the δ nitrogens of His 98 and His 103, a single carboxylate oxygen atom of Glu 60, and a solvent ligand (Fig. 2) modeled as a water molecule. The coordination geometry is best described as square pyramidal with Glu 60 sitting at the pyramid apex and the pyramid base comprising the copper ion, Cys 95, His 98, and His 103 and the water molecule. Although NC structurally resembles blue copper proteins, the molecular details of red and blue copper centers are distinct. Some blue copper centers are three-coordinate,[9] but most have one or two additional weakly bound ligands. By contrast, the red copper center has four, rather than three, strong protein ligands, with a glutamic acid oxygen 2.09 Å from the copper ion. Furthermore, the coordinated water molecule renders the red copper center five coordinate. A particularly striking difference

between red and blue copper is the Cu-S(Cys) bond distance. In blue copper, with the exception of some azurins and the subfamily of basic blue proteins, the Cu-S(Cys) distance is short, ~2.1 Å, and is responsible for the blue color.[10] The 2.26 Å distance observed in NC is similar to that observed in five-coordinate inorganic copper compounds. These differences in ligand identity and bond lengths between red and blue copper result in distinct coordination geometries.

Acknowledgments

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