Modulation of the Coordination Environment and Redox Properties of Engineered Purple Cu_A and Ferrocene Centers in Azurin

Hee Jung Hwang Final Seminar December 5, 2005

Precise control of the structure and function of proteins is often provided by metal ions or metal-containing cofactors/prosthetic groups. Recent progress in the design and engineering of metalloproteins has contributed to not only expanding our understanding of the role of metal ions in proteins but also creating new artificial metalloenzymes with enhanced or new functionalities. In the current study, two metalloproteins containing either a dinuclear Cu_A center or a ferrocene center with electron transfer function were constructed and studied (Figure 1) with the focus placed on controlling the redox properties of the metal center by modulation of the coordination environment.

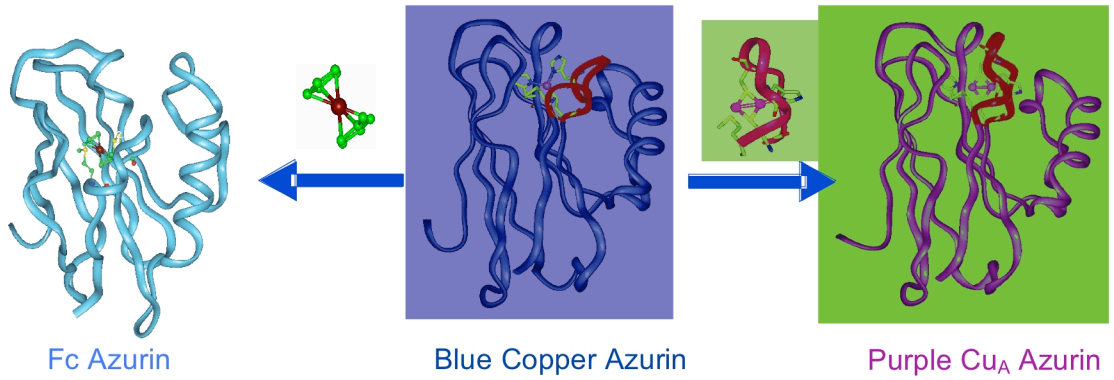

Figure 1: Engineering a ferrocene center (left) and a dinuclear Cu_A center (right) into azurin.

The Cu_A center is a mixed-valence dinuclear copper center with each copper coordinated to a histidine and both coppers bridged by the thiolate sulfurs of two cysteine ligands (Figure 2).[1] Loop-directed mutagenesis has been used to successfully engineer a Cu_A center into the protein scaffold of choice, blue copper protein azurin (Figure 1).[2] Extensive spectroscopic,[3] structural[4] and electrochemical[5] studies show that the engineered protein, Cu_A azurin, closely resembles the native Cu_A center. The effect of ligand variation on the spectroscopic and electrochemical properties of the Cu_A azurin model was investigated. When the weak, yet highly conserved axial methionine ligand was replaced with aspartate, glutamate, glutamine and leucine, small changes in the reduction potential were found. (e.g methionine to glutamate (-8 mV), aspartate (-5 mV) and leucine (+16 mV))[6] In contrast, much larger changes in the reduction potential were observed when the same mutations were made to the structurally related blue copper azurin (e.g. methionine to glutamate (-84 mV), aspartate (-14 mV) and leucine (+86 mV)). These results demonstrate the importance of the diamond core $Cu_2(Scys)_2$ structure of the Cu_A center to maintaining resistance to axial ligand variation. The two bridging cysteine ligands were also independently replaced with serine. The Cys112Ser variant has two distinct type 2 copper centers and the Cys116Ser variant contains one type 1 blue copper center with tetragonal distortion.[7]

These results show the importance of the bridging cysteine ligands in maintaining the integrity of the Cu_A center.

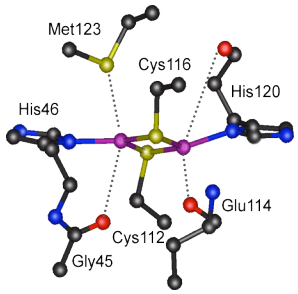

Figure 2: Active site geometry of Cu_A azurin.

The Cu_A azurin model offers a unique opportunity to deepen the understanding of mixed valence chemistry. A pH-dependent transition between delocalized and trapped mixed-valence states of an engineered Cu_A center in azurin has been investigated by UV-vis absorption and EPR spectroscopic techniques. Upon lowering the pH from 7.0 to 4.0, the absorption at 760 nm shifted to lower energy towards 810 nm, and the seven-line EPR hyperfine structure which is indicative of a delocalized state, was changed to a four-line pattern which is typical of a trapped-valence state. Lowering the pH also dramatically increased the reduction potential of the Cu_A center from 160 to 340 mV. Mutagenesis studies identified the C-terminal histidine (His120) as a site of protonation and the possible role of this histidine in regulating proton-coupled electron transfer was suggested.[8]

Engineering a metalloprotein with a non-native cofactor to create a new metalloprotein with new functionalities is of significant interest in chemistry and biology. Towards this goal, an artificial metalloprotein with a ferrocene moiety was prepared in an azurin scaffold (Figure 1) employing covalent attachment of a ferrocene derivative (2-[(Methylsulfonyl)thio]ethylferrocene to the highly conserved Cys112 residue in azurin. The resulting protein (FcAz1) exhibited an increase in the reduction potential of the ferrocene moiety from 402 mV (vs. NHE) in pH 4 aqueous buffer to 579 mV inside the protein in the same pH, consistent with the ferrocene group being encapsulated inside the hydrophobic environment of the protein. Modulation of the reduction potential of ferrocene by residues in the second coordination sphere has also been demonstrated. Raising the pH from 4 to 9 resulted in a greater than 80 mV decrease in reduction potential of the protein-bound ferrocene (from 579 to 495 mV) while replacing Met121, an amino acid residue in close proximity to ferrocene group, with a positively charged arginine or negatively charged glutamate resulted in the predicted increase or decrease in reduction potential at all pHs. Similarly, substitution of Met121 with a more hydrophobic leucine raised the reduction potential. Electrochemically oxidized FcAz1 exhibits remarkable ferrocenium stability and the practical application of the use of this protein as a redox reagent was demonstrated by the oxidation of ferro-cytochrome *c* by ferrocenium azurin.[9]

References

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