# Redox Properties of Electron Transfer Metalloproteins

*Adrian W. Bott, Ph.D.*
*Bioanalytical Systems, Inc.*
*2701 Kent Avenue*
*West Lafayette, IN*
*47906-1382*

*E-mail:*
*awb@bioanalytical.com*

*The factors that affect the redox potentials of electron transfer metalloproteins are discussed, together with specific examples.*

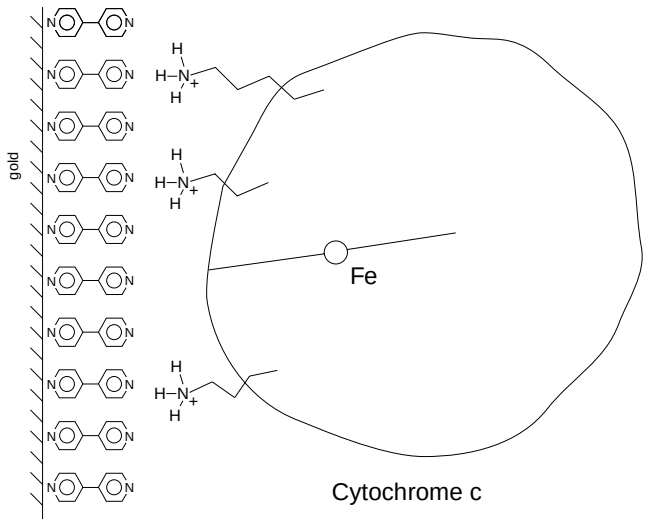
The ability of transition metals to exist in more than one stable oxidation state makes them suitable catalysts for biological processes that require transfer of electrons. Consequently, transition metals are found at the active sites of a large number of proteins. The processes catalyzed by such proteins can require the transfer of both electrons and protons to a substrate bound to the metal, or can simply involve the transfer of an electron between proteins (i.e., as part of an electron transfer chain, such as those involved in respiration or photosynthesis). Due to the physiological importance of electron transfer metalloproteins, there has been much effort devoted to understanding their electrochemical properties. The methods used for these studies, and the results obtained, are discussed in this article.

Cyclic voltammetry is widely used to characterize the redox properties of transition metal complexes. This technique can provide information about the kinetics of the electron transfer reactions and of any coupled chemical reactions, in addition to thermodynamic data (i.e., redox potentials). However, early voltammetric studies of metalloproteins were not successful (1,2). This lack of success was attributed to a number of factors, including adsorption and denaturation of the proteins at the electrode surface and inaccessibility of the coordinated metal ion (which is typically buried in the interior of the protein). Therefore, other techniques were originally used to measure the redox potentials of metalloproteins, including spectroelectrochemistry and potentiometric titration. These involve the equilibration of the oxidized and reduced forms of the analyte in a spectroscopic cell at different potentials (the potentials are determined either by addition of appropriate chemical redox reagents or by applying a constant potential to the cell). Once equilibrium has been attained, the concentrations of the oxidized and reduced species can be measured spectroscopically (e.g., using a thin-layer cell (3)). It is important to note that there is typically no direct electron transfer between the electrode surface and the analyte in spectroelectrochemical experiments; instead, small redox-active molecules (mediators) are used to transport electrons between the electrode surface and the active site of the metalloprotein. Typical mediators include quinones (4), $Ru(NH_3)_6^{3+}$ (5), and methyl viologen (6).

More recently, it has been shown that cyclic voltammograms of metalloproteins in the absence of mediators (i.e., direct electrochemistry) can be achieved under certain conditions (1,2). For example, it was shown that a quasi-reversible cyclic voltammogram could be obtained for cytochrome *c* at a gold electrode covered by a monolayer of adsorbed bipyridine (7). It was proposed that the hydrogen bonding between bipyridine molecules adsorbed to the electrode surface and lysine ($-NH_3^+$) groups on the surface of the protein in the area where the active site is exposed hold the protein close to the electrode surface in an orientation that allows rapid electron transfer between the electrode and the active site of the protein ***(F1)***. This idea of using functional groups (promoters)

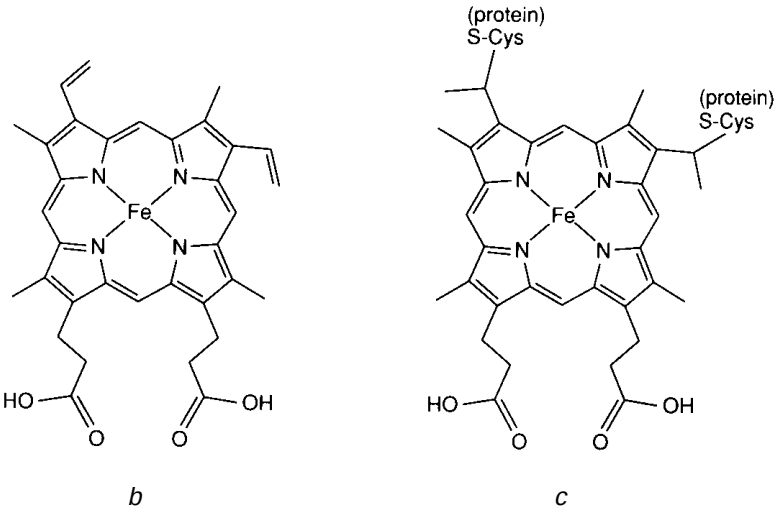
**F1**

*Schematic diagram of the interaction between the modified electrode surface and the protein that is required for electron transfer (adapted from reference 1).*




**F2**

*Molecular structures for hemes from cytochrome* b *and cytochrome* c.




on the electrode surface to bind proteins on the electrode (using electrostatic interactions) in an orientation suitable for electron transfer has been extended to other proteins (1, 2). It is important to note that the promoters are not mediators; that is, they do not act as intermediates for electron transport. It should also be noted that redox potentials measured for the same protein using the two approaches should not be expected to be the same, since the interactions between the promoter and the protein can affect the redox potential. For example, the redox potential of outer mitochondrial cytochrome $b_5$ has been measured spectroelectrochemically using both methyl viologen and $Ru(NH_3)_6^{3+}$ as mediators, and directly by cyclic voltammetry using a gold electrode modified with a mercaptopropionate/polycation complex (this complex must have a net positive charge, since cytochrome $b_5$ has a net negative charge). Redox potential values of -102 mV and -78 mV (Vs. NHE (8)), respectively, were recorded for the two methods (6).

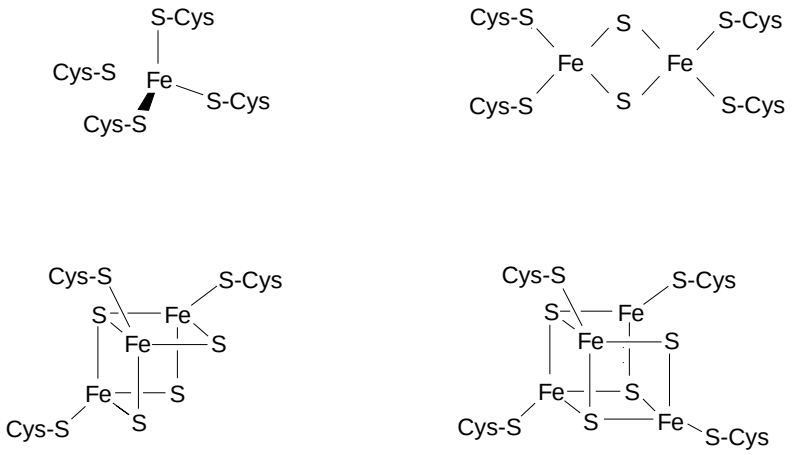
### Cytochromes

Cytochromes are part of a larger group of proteins in which a heme prosthetic group is the active site. The heme group consists of iron(III) coordinated to a porphyrin group, and the heme groups for cytochromes *b* and *c* (the two most widely studied cytochromes) are shown in ***F2***. It should be noted that the two hemes are very similar—the only difference shown in the figure is that the heme group of cytochrome *c* is coordinated to cysteine residues in the protein, whereas that of cytochrome *b* is not. However, in spite of their apparent structural similarities, the redox potentials for the iron(III)/iron(II) couple are significantly different: +5 mV for cytochrome $b_5$ (4), and +260 mV for cytochrome *c* (5). Indeed, cytochrome *c* from different sources exhibits a wide range of redox potential values (9). Consequently, a major focus of the studies of cytochromes (and other electron transfer proteins *(vide infra))* has been the investigation of the factors that affect the redox potential. In this section, only cytochrome *c* and cytochrome $b_5$ will be discussed, since these are the two best characterized cytochromes.

One method that has been used for examining the effect of structural variations on redox potentials has been the study of model compounds; that is, small molecular complexes (e.g., porphyrins (10, 11)) that contain some of the features of the active site under investigation. It should be stressed that model compounds cannot reproduce exactly the complex environment inside a protein—they are used for systematic variation of specific structural features. For example, one difference between cytochrome *c* and cytochrome $b_5$ is the axial ligation of the iron center—in cytochrome *c*, it is coordinated to one methionine residue (S coordination) and one histidine residue (N coordination), whereas in cytochrome $b_5$, it is coordinated to two histidine residues. Comparison of the redox potentials of tetraphenylporphyrin derivatives with the appropriate axial coordination (using imidazoles and thioethers for the axial ligands) shows that changing from N/N axial coordiation to N/S axial coordination leads to a shift of +170 mV (12); that is, the iron(III) oxidation state is destabilized. Another significant property of these model compounds was the insensitivity of the axial bond lengths to the oxidation state of the iron center; that is, there is little

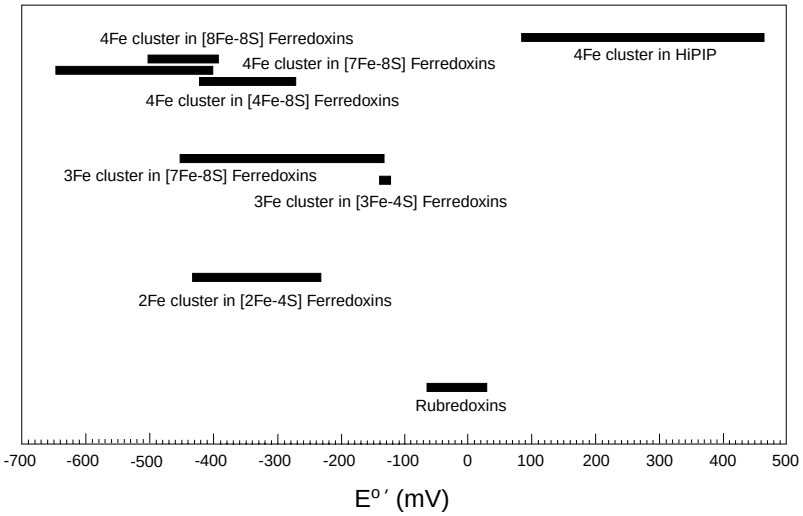
**F3**

*Molecular structures of iron sulfur prosethetic groups found in iron sulfur proteins.*




**F4**

*Range of redox potentials observed for iron sulfur proteins (relative to the normal hydrogen electrode) (adapted from reference 31).*




structural reorganization required for electron transfer, which facilitates the electron transfer reaction.

It is also possible to change specific amino acid residues in the protein to investigate the effect of this particular residue on the structure and function of the protein (site specific mutagenesis). The largest changes are obtained by changing the residue that is involved in axial coordination to the iron center (methione (Met)-80 and histidine (His)-18 for cytochrome *c*, and His-63 and His-39 for cytochrome $b_5$) (5,13-15). For example, replacement of His-39 in cytochrome $b_5$ with methionine generates a protein in which one of the axial sites is occupied by a water molecule (13). This is reflected by the shift of -240 mV in the redox potential (i.e., stabilization of the iron(III) center). It was also shown that this modified (mutant) protein could bind a substrate in the axial site and could catalyze oxidative reactions of this substrate, similar to those catalyzed by cytochrome P450 enzymes, in which there are vacant axial sites. A change in axial coordination can also be induced by changing a residue that is not directly coordinated to the iron center. Replacement of phenylalanine(Phe)-82 with histidine generates a mutant cytochrome *c* in which Met-80 and His-18 are coordinated in the iron(II) complex, whereas His-82 and His-18 are coordinated in the iron(III) state (16). Since there are two possible isomers with different redox potentials (+230 mV for the Met-80-His-18 isomer, and +150 mV for the His-82-His-18 isomer), cyclic voltammograms of this modified protein were shown by digital simulation to be consistent with a square scheme redox mechanism.

Smaller variations in the redox potentials have been found in modified versions of cytochrome *c* in which the axial coordination of the iron center is not altered (17-21). These have been attributed to changes in the following factors: the hydrophobicity of the interior of the protein, the solvent accessibility of the active site, and electrostatic and hydrogen bonding interactions between amino acid residues and the iron center (22-25). Based on comparison of the redox potentials of native cytochrome *c* with unfolded cytochrome *c* and microperoxidase (a proteolytic fragment of cytochrome *c* containing the heme group and a small number of amino acids), it has been suggested that encapsulation of the heme group in a hydrophobic environment causes a positive shift of 240 mV in the redox potential (26) (i.e., the iron(III) oxidation state is destabilized).

Since the porphyrin group in cytochrome $b_5$ is not directly bound to the enzyme, the heme group can be removed, modified, and then replaced in the active site. Modifications of the porphyrin that have been studied include esterification of the propionate groups (21,27) (which destabilizes the iron(III) state), replacement of the propionate groups with methyl groups (28) (which stabilizes the iron(III) state), replacement of the vinyl groups with alkyl groups (28,29) (which stabilizes the iron(III) state), variation of the orientation of the heme group in the active site (30), and substitution of porphyrin by chlorin analogues (31).

### *Iron-Sulfur Proteins*

There are a wide range of different iron-sulfur electron transfer proteins, which vary in the number of

iron centers (32,33). Four different iron-sulfur structures are observed (***F3***): $Fe(SR)_4^n$, $Fe_2S_2(SR)_2^n$, $Fe_3S_4(SR)_2^n$, and $Fe_4S_4(SR)_4^n$ (the bridging ligands are sulfides (formally $S^{2-}$), and the terminal ligands are $RS^-$ (where R = cysteine for protein-bound iron-sulfur clusters)). Since iron can readily exist in the +3 or +2 oxidation state, the following oxidation states are available for the $Fe_xS_y$ cluster core: +2, +1, 0 ($Fe_2S_2$); +1, 0,-1 ($Fe_3S_4$); +4, +3, +2, +1, 0 ($Fe_4S_4$). However, a given iron sulfur cluster in a protein typically only exhibits one redox process when studied by cyclic voltammetry. For $Fe_2S_2$ proteins, the two observable cluster oxidation states are +2 and +1 (2Fe(III) and Fe(III)Fe(II)), and $Fe_3S_4$ clusters can exist as +1 and 0 (3Fe(III) and 2Fe(III)Fe(II)) (although the formation of "hyper-reduced" 3Fe(II) $Fe_3S_4$ clusters has recently been reported (34)). Two different classes of iron-sulfur $Fe_4S_4$ proteins have been observed; those with oxidation states of +3 and +2 (3Fe(III)Fe(II) and 2Fe(III)2Fe(II)), and those with oxidation states of +2 and +1 (2Fe(III)2Fe(II) and Fe(III)3Fe(II)). The former group is referred to as high-potential iron-sulfur proteins (HiPIPs), whereas the latter group is referred to as ferredoxins (the term *ferredoxin* is also used to describe $Fe_2S_2$ and $Fe_3S_4$ clusters, whereas FeS proteins are referred to as rubredoxins). The range of redox potentials exhibited by iron-sulfur clusters in proteins is shown in ***F4*** (33).

Structural analyses of iron-sulfur have shown that there is little variation in the structure of the iron-sulfur clusters of a given type in different proteins (33,35). Therefore, the wide variation in measured redox potentials has again been attributed to variations in the cluster environment within the protein (e.g., hydrogen bonding, electrostatic interactions, solvent accessibility, and hydrophobicity). The different oxidation states of $Fe_4S_4$ ferredoxins and HiPIPs has been attributed to differences in the configuration of amide groups close to the clusters and the resulting cluster-amide interactions (36,37), including differences in the number of hydrogen bonding interactions (35) (it was also noted that the cluster environment in HiPIPs is more hydrophobic than it is in $Fe_4S_4$ ferredoxins (35)). The variation among $Fe_4S_4$ ferredoxins has been attributed to differences in solvent accessibility and cluster solvation (36-38), whereas the variations among HiPIPs has been attributed to variations in cluster solvation (39) and the net charge on the protein (39-41).

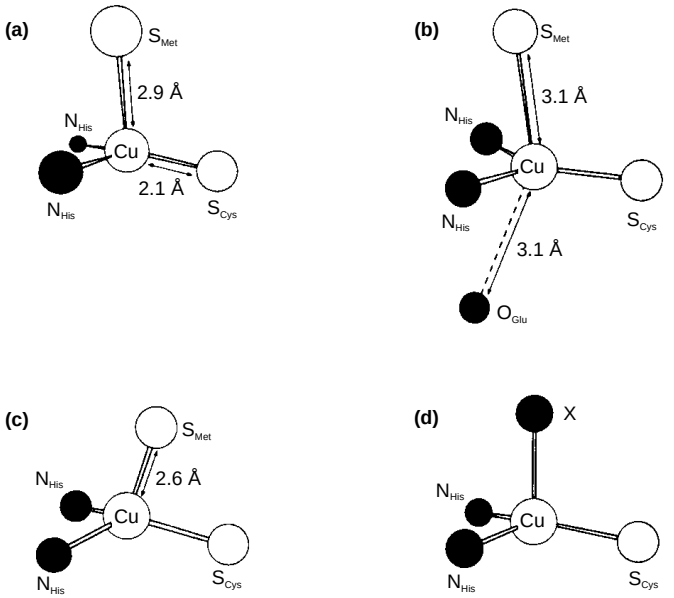
It is also possible to have two iron-sulfur clusters in the same protein. Three different classes of these proteins have been identified. "Clostridial" 8Fe ferredoxins contain two $Fe_4S_4$ clusters with similar ligating amino acid sequences (Cys-X-X-Cys-X-X-Cys-X-X-X-Cys-Pro,
where Cys = cysteine, Pro = proline, and X = other amino acid). One structurally characterized example is *Peptococcus aerogenes* ferredoxin (*Pa*Fd) (35). The redox potentials of the two clusters are essentially identical at around -400 mV (33). "Chromatial" 8Fe ferredoxins also have two $Fe_4S_4$ clusters, but the ligating sequences are different; one sequence is similar to that found in clostridial ferredoxins (the clostridial sequence), whereas in the other sequence five or six additional amino acids have been inserted between the second and third cysteines (the chromatial sequence). This structure is shown by the *Chromatium vinosum* ferredoxin (*Cv*Fd) (42). The redox potentials of these two clusters differ by about 200 mV (43) (-460 and -655 mV). In the third class, the ligating sequences are also different; one sequence is again similar to that found clostridial ferredoxins, whereas in the other sequence the second cysteine has been substituted by a different amino acid, or two additional amino acids have been inserted between the second and third cysteine. These changes decrease the number of coordinating cysteines from four to three; that is, there is one $Fe_4S_4$ cluster, and one $Fe_3S_4$ cluster (a 7Fe ferredoxin). *Azotobacter vinelandii* ferredoxin I (*Av*FdI) is one example of this type of ferredoxin (44). The redox potentials for the two clusters are significantly different (45) (-425 mV for the $Fe_3S_4$ cluster, and -650 mV for the $Fe_4S_4$ cluster).

Much effort has been devoted to rationalizing the differences in the redox potentials of the three structures described, and site-specific mutagenesis has again been extensively used. In one study, selected amino acids in *Av*FdI were changed to those at the same position in the amino acid sequence of *Pa*Fd (38) to examine the effect on the redox potential of the $Fe_4S_4$ cluster. These mutations involved substitution of three negatively charged surface amino acids for neutral ones (aspartate→asparagine, glutamate→serine, and glutamate→alanine), one neutral surface residue for a negatively charge residue (histidine→aspartate), and two hydrophobic residues in the interior of the protein (phenylalanine→tyrosine and phenylalanine→isoleucine). These amino acids were chosen because of their proximity to the $Fe_4S_4$ cluster. None of the residues had any effect on the redox potential of the $Fe_3S_4$ cluster (which is to be expected, since none of the mutations was close to this site), and only one mutation (phenylalanine→isoleucine) altered the redox potential of the $Fe_4S_4$ cluster (by about 20 mV). It was concluded from these results that surface charges had little effect on the redox potentials, and that the large difference between the redox potentials of the $Fe_4S_4$ clusters in the two proteins was associated with solvent accessibility.

A larger change in the redox potential of the $Fe_4S_4$ cluster was achieved by using a cysteine→alanine mutation at position 20 (45). Since this residue is coordinated to the cluster, this mutation forces a different coordination geometry on the peptide chain, leading to a change in the redox potential from -650 to -750 mV (as in the previous example, the

**F5**

*Molecular structures of active site of blue copper proteins. a) plastocyanin, b) azurin, c) cucumber basic protein, d) stellacyanin (adapted from reference 53).*




redox potential of the $Fe_3S_4$ cluster is not affected by this mutation). Removal of the two additional amino acids between the second and third cysteine residues of the sequence that ligates the $Fe_3S_4$ cluster (i.e., conversion to a clostridial sequence) leads to the formation of a $Fe_4S_4$ cluster rather than a $Fe_3S_4$ cluster at this site; that is, *Av*FdI has been converted from a 7Fe ferredoxin to an 8Fe ferredoxin (46). The redox potential of the new $Fe_4S_4$ cluster was found to be -466 mV. The redox potential of the other $Fe_4S_4$ cluster was -612 mV; that is, there are two $Fe_4S_4$ clusters in the same protein with similar ligating sequences, but with very different redox potentials.

As discussed above *(vide supra)*, the ligating sequences of the two $Fe_4S_4$ clusters in CvFd are different, and the redox potentials of these two centers are very different (-460 and -655 mV). Mutagenesis was used to assign the redox potentials to the two clusters; mutations around cluster I affected the more negative redox potential, whereas mutations around cluster II affected the more positive redox potential (43). One particular mutation converted the chromatial sequence around cluster II to the clostridial sequence and led to a shift in the redox potential of cluster II from -460 to -400 mV; that is, a value similar to those found for clostridial ferredoxins. However, the clostridial ligating sequence was also found for cluster I, which had a more negative redox potential. It was observed that substitution of valine by the less bulky glycine at position 13 caused a positive shift of 50 mV in the redox potential, which is consistent with the proposed role of solvent accessibility in determining redox potential (36,37).

### Blue Copper Proteins

There are a number of different coordination modes for copper in metalloproteins, and these have been labeled Type 1, Type 2, and Type 3 based on the spectroscopic properties of the protein (47,48). Type 1 copper sites are characterized by an intense blue color, due to an absorption at 600 nm, and hence proteins containing Type 1 copper are often referred to as "blue" copper proteins, whereas Type 2 copper sites have spectroscopic properties similar to those found for square-planar coordination complexes (and hence are referred to as "normal"). Type 3 copper sites contain two interacting copper centers. As discussed above, blue copper proteins are one class of electron transfer proteins.

Blue copper proteins are different from the other two classes of electron transfer proteins discussed in this article in that there is no prosthetic group associated with the copper, and the copper center is directly coordinated to amino acid residues. A number of blue copper proteins have been characterized by x-ray crystallography (47,48), and these have similar copper(II) coordination sites ***(F5)*** (although there are important differences). The common feature in all the copper sites is a trigonal planar arrangement of two N ligands (each from histidine) and one S (thiolate) ligand (from cysteine), with strong interactions between the copper center and all these ligands. The copper sites from different proteins differ in the number and strength of the axial interactions (i.e., above and below the $N_2S$ plane), and the position of the copper center relative to the $N_2S$ plane. In amicyanin (49) and plastocyanin (50) ***(F5a)***, there is a weak axial bond to a thioether (from methionine) with a Cu-S distance of about 2.90 Å, and the copper center lies 0.3 - 0.35 Å above the $N_2S$ plane (i.e., a distorted tetrahedron). In azurin (51) ***(F5b)***, there are two axial ligands, the thioether from methionine at a distance of 3.1 Å, and a carbonyl oxygen from glycine, also at a distance of 3.1 Å, with the copper center much closer (0.08 Å) to the $N_2S$ plane (i.e., a distorted trigonal bipyramid). The copper sites of cucumber basic blue protein (plantacyanin) (52) and pseudoazurin (53) ***(F3c)*** are similar to that of plastocyanin, but the axial Cu-S interaction is stronger (Cu-S = 2.62 and 2.69 Å, respectively) and there is a tetragonal distortion relative to the plastocyanin copper site that leads to differences in the spectroscopic characteristics of the copper sites (54-56). In stellacyanin (57) ***(F3d)***, the copper site is again a distorted

tetrahedron, but the axial methionine thioether has been replaced by the carbonyl oxygen of a glycine (Cu-O = 2.2 Å).

Comparison of the geometries of copper(I) sites with those of the copper(II) sites from the same protein shows that the ligand geometry does not vary with the oxidation state of the copper (58-61) (as previously mentioned, this facilitates electron transfer, since the activation energy required for structural changes is negligible). The ligand geometries also remain unaffected when the copper center is removed (62-64) or when it is substituted by mercury (65) or cadmium (66). It was concluded from these results that the geometry at the copper site is controlled by the protein, and this geometry is imposed upon the copper center (67). The unusual spectroscopic properties of blue copper proteins have been attributed to this constrained geometry (54-56). The potentials for the Cu(II)/Cu(I) redox couple are of particular interest, since they are higher (more positive) than typically observed for copper coordination complexes (68) (i.e., the copper(I) oxidation state is more favored). This behavior was explained by noting that, electronically, copper(II) favors a square planar or tetragonal geometry over a tetrahedral geometry. This is due to d orbital degeneracy in a $d^9$ tetrahedral geometry, which is removed by the tetragonal distortion (Jahn-Teller distortion) towards a square planar geometry. In contrast, the $d^{10}$ copper(I) center has no electronic preferences. Therefore, forcing the copper center into a distorted tetrahedral geometry increases the relative stability of the copper(I) oxidation state. This increased stability has been illustrated using a tetradentate imidazole ligand to constrain copper(II) in a tetrahedral geometry (69); the redox potential of this model complex was several hundred millivolts more positive than the redox potentials of $CuN_4$ complexes with a square planar geometry. However, it has recently been pointed out that there is an another Jahn-Teller distortion available for removing the degeneracy of the tetrahedral geometry; lengthening one of the bonds lowers the symmetry from $T_d$ to $C_{3v}$, and shortening one of the remaining bonds further lowers the symmetry to $C_s$ (55). This distorted geometry is achieved in the copper coordination site of plastocyanin, which has a long Cu-S bond for coordination to the methionine residue, and a short Cu-S for coordination to the cysteine residue. It was proposed that the copper(II) geometry found in plastocyanin is not a high energy structure as had been previously proposed, and therefore the geometry imposed by the protein on the metal center is not the reason for the destabilization of the copper(II) oxidation state. The high redox potential was attributed to the weak interaction between the copper center and the axial methionine ligand, since the poor electron donation from this fourth ligand increases the favorability of the lower (copper(I)) oxidation state (55).

Although the structures of the copper coordination sites are similar, there is a wide variation in the values of the redox potentials (similar to those discussed above for cytochromes and iron-sulfur proteins). Typical values include +680 mV for rusticyanin (47), +390 mV for plastocyanin (70), +320 mV for cucumber basic blue protein (71), +310 mV for azurin 72, + 280 mV for pseudoazurin (73), and +184 mV for stellacyanin (47). Considerable effort has been devoted to elucidating the factors that cause these wide variations, and, again, site-directed mutagenesis has been extensively used (74). Some selected examples are discussed below.

### *Substitution of the Amino Acids Directly Coordinated to the Copper Center*

As discussed above *(vide supra)*, the axial (fourth) ligand in most blue copper proteins is methionine (coordinated thioether), whereas in stellacyanin, it is glycine (coordinated carbonyl oxygen). The coordination of oxygen rather than sulfur may stabilize the copper(II) oxidation state and lower the redox potential. This idea has been tested by replacing the coordinating methionine (Met-121) in azurin with glutamine (75). It was shown by X-ray crystallography that the carbonyl oxygen of glutamine 121 was coordinated in the axial site. However, the redox potential only decreased by 20 mV (from +288 mV to +268 mV). This was attributed to changes in the copper coordination that occurred for the mutant protein upon reduction that stabilized the copper(I) oxidation state (similar changes did not occur in the wild type protein).

Oxygen coordination in the axial site has also been obtained for azurin by substituting Met-121 with glutamate, which can coordinate through the carboxylate group (76). However, the properties of this mutant protein are strongly dependent upon pH, due to the protonation/deprotonation of the coordinated carboxylate group. At pH 4, the carboxylate is protonated, and the spectroscopic properties are typical of a blue copper protein. However, at pH 8, the carboxylate is deprotonated, and the change in axial coordination gives rise to significant spectroscopic changes. The redox potential also changes from +370 mV at pH 4 to +184 mV at pH 8. These changes were attributed to the stronger interaction with the lone pair on the deprotonated oxygen donor. Similar behavior has also been reported for the histidine-121 mutant (77,78).

Met-121 can also be substituted by amino acids that cannot coordinate to the copper center (e.g., those with aliphatic side chains, such as glycine, valine, alanine, and leucine). These mutants typically have redox potentials higher than the wild type protein (72,79), and this has been attributed to the increased hydrophobicity of the copper coordination site (the decreased electron donation to the copper center may also need to be considered *(vide su-*

*pra))*. The vacant axial coordination site can be occupied by exogeneous ligands (e.g., water, azide, and thiocyanate); coordination of these ligands stabilizes the copper(II) oxidation state, leading to lower redox potentials (72,80,81).

The coordination sphere of copper has also been altered by substituting the histidine and cysteine amino acids that coordinate in the trigonal plane. Substitution with non-coordinating amino acids generally leads to an increase in the redox potential (82) and conversion of the copper coordination site from Type 1 to Type 2 (83,84) (as judged by changes in spectroscopic properties). Type 1 coordination can be restored by the addition of an appropriate ligand (e.g., imidazole).

### Substitution of Other Amino Acids

It has been noted in previous sections of this article that the redox potential of metal centers can be influenced by a variety of factors, including electrostatic interactions between the metal center and point charges and dipoles in the protein, hydrogen bonding between the metal center and the protein, and the hydrophobicity and solvent accessibility of the metal center. Therefore, changes in amino acids not directly coordinated to the metal center can affect the redox potential. The redox potentials of blue copper proteins can be further influenced through modulations in the tertiary structure brought about by changes in electrostatic and hydrogen bonding interactions that accompany variations in non-coordinated amino acids. The magnitude and direction of changes in the redox potential depend on the site of substitution and changes in charge, polarity, etc. (72,74). Since there are so many variables to consider, structural studies on the mutant proteins are generally required when trying to rationalize changes in redox potentials. For example, the solid state structures of two mutants of pseudoazurin (proline-40 substituted by alanine or isoleucine) in both oxidation states were elucidated using X-ray crystallography (85). These showed that the substitution by alanine created a surface pocket containing a water molecule, and electrostatic calculation showed that the presence of this additional water molecule altered the solvation energy at the copper center. The substitution by isoleucine created more protein flexibility at the copper binding site that allowed a more trigonal geometry to be adopted by the copper(I) oxidation state. Both these changes stabilize the copper(I) oxidation state, which is consistent with the increase in the redox potential from +270 mV for the wild type protein to +409 mV in the alanine mutant and +450 mV in the isoleucine mutant.

The examples given above for the three different classes of electron transfer proteins illustrate how the protein environment is used to modulate the redox potentials over a wide range. However, although much progress has been made in determining the effects of protein environment, considerable work is still required (i.e., relating changes in the redox potential to changes in the rate of electron transfer between proteins (86)).

### References


1. *F.A. Armstrong, H.A.O. Hill, and N.J. Walton, Acc. Chem. Res. 21 (1988) 407.*
2. *F.A. Armstrong, Struct. Bond, 72 (1990) 137.*
3. *W.R. Heineman, C.W. Anderson, H.B. Halsall, M.M. Hurst, J.M. Johnson, G.P. Kreishman, B.J. Norris, M.J. Simone, and C.-H. Su in "Electrochemical and Spectroelectrochemical Studies of Biological Redox Components" (K.M. Kadish, ed.), ACS Adv. Chem. Ser. 201 (1982) 1.*
4. *L.S. Reid, V.T. Taniguchi, H.B. Gray, and A.G. Mauk, J. Am. Chem. Soc. 104 (1982) 7516.*
5. *A.L. Raphael and H.B. Gray, J. Am. Chem. Soc. 113 (1991) 1038.*
6. *M. Rivera, M.A. Wells, and F.A. Walker, Biochemistry 33 (1994) 2161.*
7. *M.J. Eddowes and H.A.O. Hill, J. Chem. Soc., Chem. Commun. (1977) 3154.*
8. *All potentials quoted in this article are relative to the NHE.*
9. *M.A. Cusanovich, T.E. Meyer, and G. Tollin in "Advances in Inorganic Biochemistry: Heme Proteins" G.L. Eichhorn and L.G. Marzelli (eds.), Elsevier (1988) 37.*
10. *L.A. Bottomley, L. Olsen, and K.M. Kadish in "Electrochemical and Spectroelectrochemical Studies of Biological Redox Components" (K.M. Kadish, ed.), ACS Adv. Chem. Ser. 201 (1982) 279.*
11. *F.A. Walker, J.A. Barry, V.L. Balke, G.A. McDermott, M.Z. Wu, and P.F. Linde in "Electrochemical and Spectroelectrochemical Studies of Biological Redox Components" (K.M. Kadish, ed.), ACS Adv. Chem. Ser. 201 (1982) 378.*
12. *T. Mashiko, C.A.Reed, K.J. Haller, M.A. Kastner, and W.R. Scheidt, J. Am. Chem. Soc. 103 (1981) 5758.*
13. *S.G. Sligar, K.D. Egeberg, J.T. Sage, D. Morikis, and P.M. Champion, J. Am. Chem. Soc. 109 (1987) 7896.*
14. *C.J.A. Wallace and I. Clark-Lewis, J. Biol. Chem. 267 (1992) 3852.*
15. *J.C. Rodriquez and M. Rivera, Biochemistry 37 (1998) 13082.*
16. *B.A. Feinberg, X. Liu, M.D. Ryan, A. Schejter, C. Zhang, and E. Margoliash, Biochemistry 37 (1998) 13091.*
17. *S.P. Rafferty, L.L. Pearce, P.D. Barker, J.G. Guillemette, C.M. Kay, M. Smith, and A.G. Mauk, 29 (1990) 9365.*
18. *W.D. Funk, T.P. Lo, M.R. Mauk, G.D. Brayer, R.T.A. MacGillivray, and A.G. Mauk, Biochemistry 29 (1990) 5500.*
19. *K.K. Rodgers and S.G. Sligar, J. Am. Chem. Soc. 113 (1991) 9419.*
20. *A.L. Burrows, L.-H. Guo, H.A.O. Hill, G. McLendon, and F. Sherman, Eur. J. Biochem. 202 (1991) 543.*
21. *M. Rivera, R. Seetharaman, D. Girdhar, M. Wirtz, X. Zhang, W. Wang, and S.White, Biochemistry 38 (1998) 1485.*
22. *R.J. Kassner, Proc. Nat. Acad. Sci. USA 69 (1972) 2263.*
23. *A.K. Churg and A. Warshel, Biochemistry 25 (1986) 1675.*
24. *G.R. Moore, G.W. Pettigrew, and N.K. Rodgers, Proc. Natl. Acad. Sci 83 (1986) 4998.*
25. *A.G. Mauk and G.R. Moore, J. Biol. Inorg. Chem. 2 (1997) 119.*
26. *F.A. Tezcan, J.R. Winkler, and H.B. Gray, J. Am. Chem. Soc. 120 (1998) 13383.*
27. *L.S. Reid, M.R. Mauk, and A.G. Mauk, J. Am. Chem. Soc.106 (1984) 2182.*
28. *K.-B. Lee, E. Jun, G.N. La Mar, I, Rezzano, R.K. Pandey, K.M. Smith, F.A. Walker, and D.H. Buttlaire, J. Am. Chem. Soc. 113 (1991) 3576.*

29. L.S. Reid, A.R. Lim, and A.G. Mauk, J. Am. Chem. Soc. 108 (1986) 8197.
30. F.A. Walker, D. Emrick, J.E. Rivera, B.J. Hanquet, and D.H. Buttlaire, J. Am. Chem. Soc. 110 (1988) 6234.
31. S.A. Martinis, C. Sotiriou, C.K. Chang, and S.G. Sligar, Biochemistry 28 (1989) 879.
32. R. Cammack, Adv. Inorg. Chem. 38 (1992) 281.
33. F. Capozzi, S. Curli, and C. Luchinat, Struct. Bonding 90 (1998) 127.
34. J.L.C. Duff, J.L.J. Breton, J.N. Butt, F.A. Armstrong, and A.J. Thomson, J. Am Chem. Soc. 118 (1996) 8593.
35. G. Backes, Y. Mino, T.M. Loehr, T.E. Meyer, M.A. Cusanovich, W.V. Sweeney, E.T. Adman, and J. Sanders-Loehr, J. Am. Chem. Soc. 113 (1991) 2055.
36. R. Langen, G.M. Jensen, U. Jacob, P.J. Stephens, and A. Warshel, 267 (1992) 25625.
37. G.M. Jensen, A. Warshel, and P.J. Stephens, Biochemistry 33 (1994) 10911.
38. B.Shen, D.R. Jollie, C.D. Stout, T.C. Diller, F.A. Armstrong, C.M. Gorst, G.N. La Mar, P.J. Stephens, and B.K. Burgess, J. Biol. Chem. 269 (1994) 8564.
39. H.A. Heering, Y.B.M. Bulsink, W.R. Hagen, and T.E. Meyer, Biochemistry 34 (1995) 14675.
40. L. Banci, I. Bertini, S. Curli, C. Luchinat, and R. Pierattelli, Inorg. Chim. Acta. 24 (1995) 251.
41. L. Banci, I. Bertini, G.G. Savellini, and C. Luchinat, Inorg. Chem. 35 (1996) 4248.
42. J.M. Moulis, L.C. Sieker, K.S., Wilson, and Z. Dauter, Protein Sci. 5 (1996) 1765.
43. P. Kyritsis, O.M. Hatzfeld, T.A. Link, and J.M. Moulis, J. Biol. Chem. 273 (1998) 15404.
44. C.D. Stout, J. Mol. Biol. 205 (1989) 545.
45. S.E. Iismaa, A.E. Vazquez, G.M. Jensen, P.J. Stephens, J.N. Butt, F.A. Armstrong, and B.K. Burgess, J. Biol. Chem. 266 (1991) 21563.
46. M.A. Kemper, H.S. Gao-Sheridan, B. Shen, J.L.C. Duff, G.J. Tilley, F.A. Armstrong, and B.K. Burgess, Biochemistry 37 (1998) 12829.
47. A.G. Sykes, Adv. Inorg Chem. 36 (1991) 377.
48. A. Messerschmidt, Struct. Bonding 90 (1998) 37.
49. R. Durley, L. Chen, L.W. Louis, F.S. Mathews, and V.I. Davidson, Protein Sci. 2 (1993) 739.
50. J.M. Guss and H.C. Freeman, J. Mol. Biol. 169 (1983) 521.
51. E.N. Baker, J. Mol. Biol. 203 (1988) 1071.
52. J.M. Guss, E.A Merritt, R.P. Phizackerley, B. Hedman, M. Murata, K.O. Hodgson, and H.C. Freeman, Science 241 (1988) 806.
53. E.T. Adman, S. Turley, R. Bramson, K. Petratos, D. Banner, D. Tsernoglou, T. Beppu, and H. Watanbe, J. Biol. Chem. 264 (1989) 87.
54. R.H. Holm, R. Kennephol, and E.I. Solomon, Chem. Rev. 96 (1996) 2239.
55. J.A. Guckert, M.D. Lowery, and E.I. Solomon, J. Am. Chem. Soc. 117 (1995) 2817.
56. L.B. LaCroix, D.W. Randall, A.M. Nersissian, C.W.G. Hoitink, G.W. Canters, J.S. Valentine, and E.I. Solomon, J. Am. Chem. Soc. 120 (1998) 9621.
57. P.J. Hart, A.M. Nersissian, R.G. Herrmann, R.M. Nalbandyan, J.S. Valentine, and D. Eisenberg, Protein Sci. (1996) 2175.
58. W.E.B. Shepard, B.F. Anderson, D.A. Lewandowski, G.A. Norris, and E.B. Baker, J. Am. Chem. Soc. 112 (1990) 7817.
59. J.M. Guss, P.R. Harrowell, M, Murata, V.A. Norris, and H.C. Freeman, J. Mol. Biol. 192 (1986) 361.
60. E. Vakoufari, K.S. Wilson, and K. Patratos, FEBS Lett. 347 (1994) 203.
61. C.A.P. Libeu, M. Kukimoto, M. Nishiyama, S. Horinouchi, and E.T. Adman, Biochemistry 36 (1997) 13160.
62. T.P.J. Garrett, D.J. Clingeleffer, J.M. Guss, S.J. Rodgers, and H.C. Freeman, J. Biol. Chem. 259 (1984) 2822.
63. W.E.B. Shepard, R.L. Kinston, B.F. Anderson, and E.N. Baker, Acta Crystallogr. D49 (1993) 331.
64. K.Z. Petratos, M. Papadovasilaki, and Z. Dauter, FEBS Lett. 368 (1995) 442.
65. W.B. Church, J.M. Guss, J.J. Potter, and H.C. Freeman, J. Biol. Chem. 261 (1986) 234.
66. K.A. Blackwell, B.F. Anderson, and E.N. Baker, Acta Crystallogr. D50 (1994) 263.
67. G. Malmström. Eur. J. Biochem. 223 (1994) 711.
68. N. Kitajima, Adv. Inorg. Chem. 39 (1992) 1.
69. S. Knapp, T.P. Kennan, Z. Xioahua, R. Fikar, J.A. Potenza, and H.J. Schugar, J. Am. Chem. Soc. 112 (1990) 3452.
70. F.N. Büchi, A.M. Bond, R. Codd, L.N. Huq, and H.C. Freeman, Inorg. Chem. 13 (1992) 5007.
71. G. Battistuzzi, M. Borsari, and M. Sola, J. Inorg. Biochem. 69 (1998) 97.
72. T. Pascher, B.G. Karlsson, M. Nordling, B.G. Malmström, and T. Vanncard, Eur. J. Biochem. 212 (1993) 289.
73. T. Sakurai, O. Ikeda, and S. Suzuki, Inorg. Chem. 29 (1990) 4715.
74. G.W. Canters and G. Gilardi, FEBS Lett. 325 (1993) 39.
75. A. Romero, C.W.G. Hoitkin, H. Nar, R. Huber, A. Messerschmidt, and G.W. Canters, J. Mol. Biol. 229 (1993) 1007.
76. B.G. Karlsson, L.C. Tsai, H. Nar, J. Sanders-Loehr, N. Bonander, V. Langer, and L. Sjolin, Biochemistry 36 (1997) 4089.
77. S.J. Kroes, C.W. G. Hoitink, and G.W. Canters, Eur. J. Biochem. 240 (1996) 342.
78. A. Messerschmidt, L. Prade and G.W. Canters, Proc. Natl.Acad. Sci. USA 95 (1998) 3443.
79. A.J. Di Bilio, T.K. Chang, B.G. Malmström, H.B. Gray, B.G. Karlsson, M. Nordling, T. Pascher, and L.G. Lundberg, Inorg. Chim. Acta 198-200 (1992) 145.
80. M. Vidakovic and J.P. Germanas, Angew. Chem., Intl. Eng. Ed. 34 (1995) 1622.
81. L.M. Murphy, R.W. Strange, B.G. Karlsson, L.G. Lundberg, T. Pascher, B. Reinhammar, and S.S. Hasnain, Biochemistry 32 (1993) 1965.
82. G. van Pouderoyen, C.R. Andrew, and G.W. Canters, Biochemistry 35 (1996) 1397.
83. T. den Blaauwen, and G.W. Canters, J. Am. Chem. Soc. 115 (1993) 1121.
84. T.J. Mizoguchi, A.J.Di Bilio, H.B. Gray, and J.H. Richards, J. Am. Chem. Soc. 114 (1992) 10076.
85. C.A.P. Libeu, M. Kukimoto, M. Nishiyama, S. Horinouchi, and E.T. Adman, Biochemistry 36 (1997) 13160.
86. J.K. Hurley, A.M. Weber-Main, M.T. Stankovich, M.W. Benning, J.B. Thoden, J.L. Vanhooke, H.M. Holden, Y.K. Chae, B. Xia, H. Cheng, J.L. Markley, M. Martinez-Julez, C. Gomez-Moreno, J.L. Schmeits, and G. Trolin, Biochemistry 36 (1997) 11100.