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On the Relationship between Protein-Forced Ligand Fields and the Properties of Blue Copper Centers

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On the Relationship between Protein-Forced Ligand Fields and the Properties of Blue Copper Centers

The stable conformation of blue copper proteins forces the metal ion into a unique geometrical ligand arrangement. Spectroscopic data suggest that at least 70 $kJmol^{-1}$ would be required to twist the protein into a conformation that would allow the cupric ion to occupy a square planar site. The observation that the $\sigma S \rightarrow Cu(x^2 - y^2)$ transition energy does not change with increasing ligand field strength is interpreted in terms of a model in which Cu(*xz,yz*)-ligand back bonding enhances the splitting between the $Cu(x^2 - y^2)$ and Cu(*xz,yz*) levels in blue sites with high chemical reduction potentials, thereby providing an explanation of the special stability of the cuprous state.

In 1954 the idea of a "rack mechanism" was introduced[1,2] to explain alterations in the reactivity of amino acid side chains and metal–ion prosthetic groups in the active sites of enzymes and other biologically active proteins. According to this hypothesis, key functional groups are distorted by the overall protein conformation, thereby leading to anomalous properties. Experimental support for this concept was soon obtained from investigations on metalloenzymes[3,4]; in particular, it was found that a number of copper proteins active in electron transfer reactions were characterized by unique spectroscopic parameters.[4] The cupric sites with unusual EPR characteristics, now known as type 1 copper, found in laccase and ceruloplasmin[4] as well as in some small copper proteins such as azurin,[5] could not, according to the coordination chemistry of the time, account for the intense blue color of these proteins. Consequently, Williams[6] suggested that all intensely blue copper proteins contain cuprous ion. This was, however, excluded by the finding of blue proteins with a single type 1 cupric ion,[5,7] and also by

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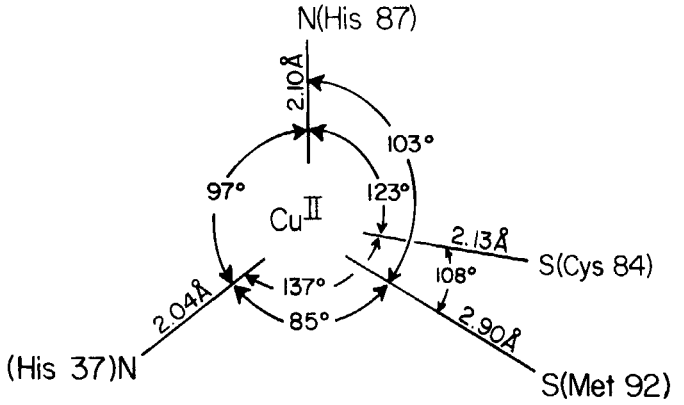
experimental work on the multicopper oxidases.[8] Thus, the idea that the anomalous properties were associated with a cupric ion in a strained configuration gained support.

Extensive comparative work on the structural, spectroscopic, thermodynamic and kinetic properties of the type 1 copper ions in a large number of proteins[9] has made it possible to perform more concrete and quantitative analyses of the rack mechanism as a basis for the unique coordination properties of metalloproteins. In this Comment we will estimate the minimum protein conformational energy that is required to force cupric ion to adopt the special structure of a blue site, and then we will discuss how this distorted structure is related to the spectroscopic properties and the reduction potentials of the proteins.

In our analysis of site geometry we shall concentrate on plastocyanin, whose structure in the vicinity of the copper is known in detail[10] (Figure 1). The relatively low *d–d* transition energies for plastocyanin demonstrate that the blue copper is severely ligand field (LF) destabilized by the geometry that is forced upon it by the protein. A minimal model for the calculation of this CuN_2SS^* site destabilization need only include the $Cu(x^2-y^2)$ and $Cu(xz,yz)$ antibonding levels. Because the $Cu(x^2-y^2)$–$Cu(xz,yz)$ splitting would be at least 9000 cm^{-1} greater for square planar CuN_2SS^* than for the blue copper in plastocyanin,[9,11] the minimum blue site electronic destabilization is 2/3(9000 cm^{-1}) = 6000 cm^{-1}. Inclusion of the $Cu(z^2)$ and $Cu(xy)$ orbitals in the calculation would not change this result very much, because it has been shown that the antibonding character of these orbitals does not vary significantly upon conversion of a blue copper site to planar coordination. It may be estimated that the relatively small correction for this added electronic effect would just offset the minor difference in interligand repulsions between a blue site and a planar CuN_2SS^* unit. Thus it is not likely that any new light will be shed on the matter of site energetics by extending the calculation in a formal way to include these two minor effects.

It is clear, from the observation that the plastocyanin binding site is not distorted significantly upon incorporation of copper in the apoprotein,[10] that the lowest energy of a protein conformation that would accommodate a planar CuN_2SS^* unit must be over 70 $kJmol^{-1}$ (~6000 cm^{-1}) above that of the observed conformation. Put in another way, the energy that would be released upon formation of an optimally LF-stabilized CuN_2SS^* unit falls well below that required to twist the protein



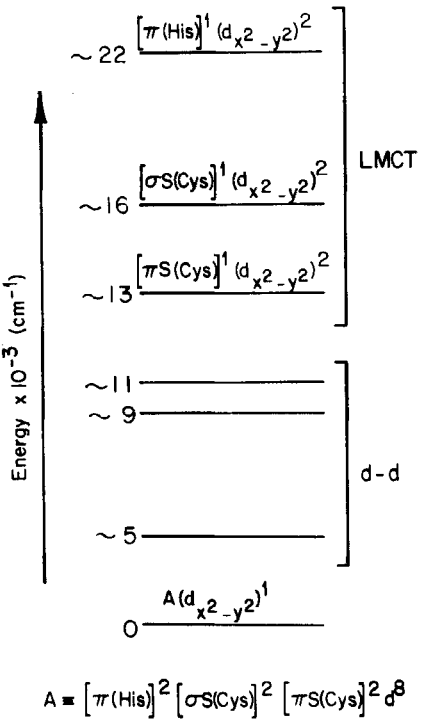


FIGURE 1. Structure and electronic energy levels of the CuN_2SS^* unit in plastocyanin (N = His; S = Cys; S* = Met).

accordingly, with the result that the copper ion is forced to accept the geometrical structure that is presented to it. Both the high reduction potentials and the facile outer sphere electron transfer kinetic properties of blue copper centers[9] are directly related to this protein-forced LF-destabilized site structure.

But, we may ask at this point, just how does the copper ligand field tune the reduction potentials in blue proteins? In considering our answer to this question, it is worth remembering that the energy of the $\sigma S \rightarrow Cu(x^2-y^2)$ charge transfer transition does not vary significantly from protein to protein, whereas the standard chemical reduction potential does.[9] An electronic structural model that accommodates this remarkable finding is shown in Figure 2. The key to this model is the introduction of a ligand–copper interaction that allows for a variation in π back bonding (Cu $\rightarrow$ L), thereby creating a higher ligand field for strong π interactions and at the same time conferring special stabilization to the cuprous state.

The importance of π back bonding in the blue copper unit is evidenced

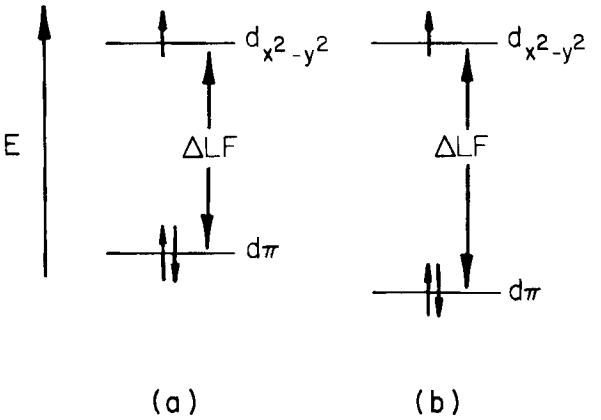


FIGURE 2. Electronic structural model for blue copper centers in which there is a variation of ligand π-acceptor interactions (for simplicity only one component (designated $d\pi$) of Cu(*xz,yz*) is shown): (a) Relatively weak π-acceptor ligands, as in stellacyanin; (b) strong π-acceptor ligands, as in fungal laccase. The π-acceptor ligand environment of site (b) more readily accommodates the electron that is added in forming copper(I), owing to preferential stabilization afforded by back bonding from the four Cu(*xz,yz*) electrons.

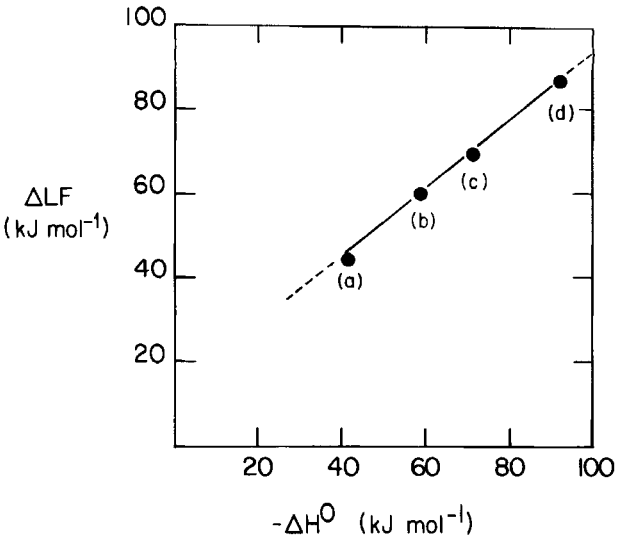


FIGURE 3. Plot of ΔLF vs. $-\Delta H^\circ$ for stellacyanin(a), plastocyanin(b), azurin(c) and fungal laccase(d).

by the linear correlation of increasing ΔLF[11] with decreasing electron transfer reaction enthalpy[12,13] (Figure 3). In the framework of our electronic structural model both ΔLF and ΔH° are related to the degree of antibonding in the Cu*(xz,yz)* level. Stabilization of Cu*(xz,yz)* by back bonding produces a larger ΔLF as well as a relatively more stable cuprous state, thereby explaining the correlation with ΔH° values. The ligand or ligands that participate in back bonding tuning of the blue copper spectroscopic and thermodynamic parameters are not known, but at this time two possibilities appear to us to be reasonable. One is related to the relatively weak copper–thioether bond in plastocyanin[9,10]; specifically, replacement of that interaction with a poorer π back bonding ligand (e.g., a disulfide,[11] or in the extreme, no fourth ligand at all[12]!) would decrease ΔLF and $-\Delta H^\circ$ (as in stellacyanin), and a better π back bonder (e.g., a normal copper–thioether) would increase these parameter values (as in fungal laccase[12]). An alternative that also appears to be attractive is one in which NH–S hydrogen bonding affects π back bonding in the blue copper unit. An interaction of this type has been

identified in the plastocyanin structure,[10] and a variation in the interaction would be expected to influence ΔLF and ΔH° to some extent.

Additional evidence that there are significant variations in copper–ligand binding interactions in the various blue copper sites may be extracted from resonance Raman spectra. Although the detailed assignments of the Cu–L vibrations that give rise to the intense peaks in the 400-cm^{-1} region are still the subject of debate, it is heartening that these vibrations (at least some of which must correspond to Cu–L stretching) shift to higher energies as the ligand fields strengthen (stellacyanin < plastocyanin < fungal laccase).[14] Thus the basis for our model, namely, the recognition that higher reduction potentials can be related to higher ligand fields, seems secure.

In closing we emphasize the key points we have put forward in this essay. The protein-forced distorted site geometry of blue copper centers not only is responsible for the high reduction potentials and facile electron transfer kinetics, but apparently also provides one attractive way to tune the potentials further. Our finding that in at least certain key cases the variation in reduction potentials is enthalpically dominated and is further connected to the ligand field strength of the site indicates strongly that π back bonding plays a crucial role in the electronic structure of blue copper. We have singled out the Cu(*xz,yz*) electrons for manipulation through π back bonding interactions because they are the ones that have suffered most through acquisition of antibonding character in the protein-forced blue site geometry.

Finally, we wish to point out that other reduction-potential tuning mechanisms clearly are possible for blue copper proteins. Indeed, it is not likely that entropic factors (related to solvation effects and conformational changes upon electron transfer) always will play a minor role in determining these potentials. The point of our essay is that site electronic effects *can* tune blue copper potentials, not that they always will. In this respect the importance of making appropriate connections between spectroscopic and thermodynamic data in evaluating site properties can hardly be overestimated.

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