Structural Features and Biological Functions in Blue Copper Proteins

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A new idea that elucidates the electron carrier ability of plastocyanin (and of azurin) is proposed. It emphasizes the fact that two lobes of the d-orbital, where one unpaired electron of copper (II) ion lies, are not screened by the ligand atoms, which would facilitate the electron transfer between the d-orbital and the redox partners (cytochrome *f* and P-700 in the case of plastocyanin). Several evidences which support the above proposal are provided.

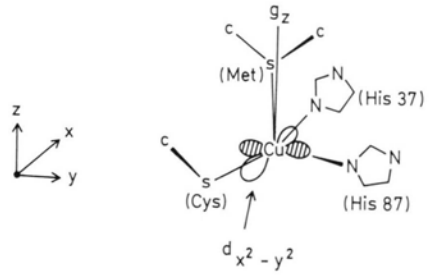
Introduction

The blue copper active site is found in a number of proteins containing single copper centers, including plastocyanins, azurins, and stellacyanins [1, 2]. This site is also found in the multicopper oxidases: laccase, ceruloplasmin, and ascorbate oxidase. These sites are distinguished by an intense absorption band ($\varepsilon \sim 10^3 \times 10^4$ units) near 600 nm and on abnormally narrow hyperfine splitting ($|A_{//}|$) in their ESR spectrum. In these proteins where the function of the blue copper site has been clearly determined, it participates in "outer-sphere electron transfer reaction". There are, however, few studies on the goal of relating the unusual electronic structure to its biological functions, although crystal data are available for several cases [3, 4].

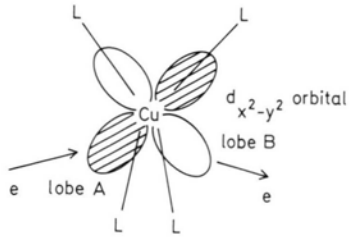
Recently the author has determined the crystal structures of several model compounds for the blue copper site [5], and concluded [5] that the geometry around the copper (II) ion in plastocyanin should be considered to be C_{3v} (trigonal) [6], and succeeded in elucidating the origin for the narrow $|A_{//}|$ values [7]. In this article we will present the new idea that relates the structural feature (C_{3v} of plastocyanin) and biological function of the blue copper proteins.

New Model

According to Penfield *et al.* [8], the one unpaired electron lies in the $d_{x^2-y^2}$ orbital (see below) in the plastocyanin, and the tensor axis of g_z is nearly parallel to the Cu-S (thioether) bonding. The present author would like to point out that the electron transfer ability of plastocyanin can be closely related to the fact that two lobes of the $d_{x^2-y^2}$ orbital are not screened by the ligand atoms (see below); so that the



absence of shielding of the d-orbital would greatly facilitate the electron transfer reaction between the d-orbital and the orbitals of redox partners (cytochrome *f* and P-700 in the case of plastocyanin), because the very small overlap between the orbitals of redox partners would make the electron transfer very easy. If the copper (II) ion accepts the electron at the lobe A site and releases it at the lobe B site (see below), the activation energy for this reaction should be very low because no structural change is needed.



L: ligand atom; e: electron

Several experimental evidences for the above discussion will be presented.

Nishida *et al.* [9] showed that the distorted tetrahedral copper (II) complexes such as $[Cu(sal\text{-}R)_2]$ (see below) exhibit quite different reactivities from those of square planar copper (II) complexes such as [Cu(salen)] in the catalytic activities for (1) the decomposition of hydrogen peroxide and (2) the oxidation reaction of TMPD by the O_2 molecule. As the $d_{x^2-y^2}$ orbital containing one unpaired electron is not

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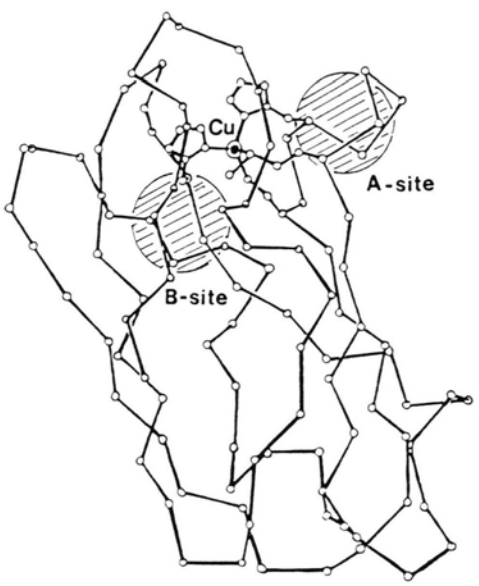
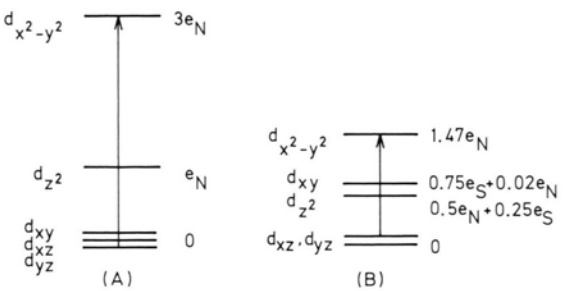
completely screened by the ligand atoms in these tetrahedral copper (II) complexes [9], we concluded that the unique reactivity of the tetrahedral complexes can be attributed to the absence of shielding of the d-orbital. These facts indicate that the d-orbital without shielding by the ligand atoms displays the high affinity for the coordination of another molecule, and also for the acceptance of an electron.

It is well known that plastocyanin functions as an electron carrier from cytochrome *f* to P-700 in the photosynthetic system [10]. There are many evidences that cytochrome *f* binds with plastocyanin at the hydrophobic site of Pro-85 and Ala-90 [11], the region being illustrated as A-site in the following figure. On the other hand, P-700 binds to plastocyanin at the electrostatic site (B-site in the figure below) of Glu-59, Glu-60, and Asp-61 [12]. It should be noted



here that these two sites are near the places where the two lobes of the $d_{x^2-y^2}$ orbital spread. This is consistent with our proposal.

If we assume C_{3v} symmetry for the copper (II) ion in plastocyanin (neglecting the bonding between copper (II) ion and sulfur of methionine), the splitting scheme of the d-orbitals can be calculated in terms of the conventional angular overlap model [13] as shown in (B) of the figure below where an interaction between copper and sulfur of the thioether group is neglected [5]. For comparison, the result obtained for $[Cu(imidazole)_4]^{2+}$ is also illustrated in (A). The d-d bands of $[Cu(imidazole)_4]^{2+}$ are observed in the range $13-18\times10^3$ cm^{-1}, and thus the parameter e_N (see below) may be calculated to be $\leq 6\times10^3$ cm^{-1}, leading the highest d-d band of



8.8×10^3 cm^{-1} for plastocyanin. This is consistent with the result of Solomon *et al.*

If the interaction between copper and the sulfur atom of the thioether is strong, the order of the split d-orbitals must change as $d_{z^2} > d_{x^2-y^2} \sim d_{xy} > d_{xz} \sim d_{yz}$. This case corresponds to trigonal bipyramidal complexes, and the unpaired electron is localized in the d_{z^2} orbital which is perpendicular to $d_{x^2-y^2}$ orbital. This situation, however, is not suitable for electron transfer ability of plastocyanin, as mentioned above. In other words, no bonding between copper (II) ion and the methionine sulfur atom is necessary for the emergence of the function of plastocyanin.

The same discussion can be applied to azurin. Very recently Norris *et al.* [4] reported the crystal structure of azurin of species, and concluded that the copper (II) ion is of a trigonal bipyramidal structure. However, it should be noted that the bond distances between copper (II) ion and two atoms at the apical positions are very long (3.13 Å for Cu-S (thioether); 3.14 Å for Cu-oxygen atom). This situation is very similar to that observed for plastocyanin as described in this article. Thus, we may understand the function of azurin in terms of the principle proposed for plastocyanin.

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