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Review article

Blue Copper Proteins: A rigid machine for efficient electron transfer, a flexible device for metal uptake

Sergio Alejo Pérez-Henarejos [a], Luis A. Alcaraz [b], Antonio Donaire [a, *]

[a] Department of Inorganic Chemistry, Faculty of Chemistry, University of Murcia, Campus Universitario de Espinardo, 30100 Murcia, Spain
[b] Bioarray SL, Alicante, Parque Científico de la UMH, Avda Universidad s/n, 03202 Elche, Alicante, Spain

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ABSTRACT

Blue Copper Proteins (BCPs) are small and generally soluble copper-containing proteins which participate in monoelectron transfer processes in biological systems. An overview of their electronic and tertiary structure is detailed here. The well-established entatic/rack-induced mechanism is explained by comparing thermodynamic parameters between the folded (tense) and the unfolded (relaxed) forms of the BCP rusticyanin.

Recently, NMR solution data have shown that the active sites of BCPs in absence of the metal ion, *i.e.* in the apoforms, are flexible in the micro-to-second timescale. The rigidity proposed by the entatic/rack-induced mechanism is an imperative for the holoprotein to perform electron transfer; while the flexibility of the apocupredoxin is necessary to uptake the metal ion from the metallochaperones. These apparently contradictory requirements are discussed in the present work. Finally, the role of azurin and some peptides derived from it in anticancer therapy are also described.

Contents

Abbreviations: AADH, amine dehydrogenase; Am, amicyanin; Az, azurin; BCPs, Blue Copper Proteins; CPP, cell penetrating peptides; CSP, chemical shift perturbation; EPR, electronic paramagnetic resonance; ET, electron transfer; LMCT, ligand to metal charge transfer; MADH, methylamine dehydrogenase; $MSP1_{19}$, the 19-kDa C-terminal fragment of the *Plasmodium* merozoite surface protein-1; NMR, nuclear magnetic resonance; Pc, plastocyanin; PDB, protein data bank; PSI, phosystem I complex; Rc, rusticyanin; rmsd, root mean squared deviation; ROS, reactive oxidative species; TTQ, tryptophan tryptophylquinone; wt, wild type.

* Corresponding author.
E-mail address: adonaire@um.es (A. Donaire).

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1. Copper in biological systems

Copper is a first-row transition metal which is essential for life. Although it is only located in low concentration in the Earth crust (*ca*. 60 ppm), it is present in almost all living organisms. The quantity of copper in healthy humans is estimated to be around 100–150 mg. Its deficiency can produce grave illness and, if it is acute, death [1–3].

Copper is very versatile: it can be found in nature with oxidation states I, II or intermediate mixed valences. Since the interconversion between the redox states I and II is easily accessible, copper has been chosen by the evolution as one of the two acceptable ions to perform electron transfer (ET) processes (being iron the other chosen one) [4,5]. Copper(II), when combined with oxygen and nitrogen donor atoms, usually becomes soluble and hence, accessible for biological systems. On the contrary, highly insoluble copper(I) has a tendency to coordinate softer donor atoms, such as sulfur. Consequently, when copper(I) combines with these atoms its solubility is extraordinarily increased. Thus, the pair copper(II)/copper(I), and its mixed valence combinations, are optimal candidates to participate in electron transfer processes [6–8]. It is remarkable that copper(I) is a d^{10} ion and so its complexes are diamagnetic and colorless; copper(II), with an uncompleted d^9 shell, provides its complexes with singular spectroscopic features. As a result, most of the knowledge of the electronic structure, and therefore, the function of copper proteins, is due to the studies performed in the paramagnetic oxidized copper(II) form [8,9].

Copper(II) complexes have been classified in different types [10], namely: type 1, type 2, type 3, CuA, CuB and CuZ (Fig. 1).

Type 1 or blue copper centers (see Fig. 2) give rise to, among others, the Blue Copper Proteins (BCPs), also called cupredoxins, a special set of proteins that are the matter of the present revision. Their features will be extensively commented in the following sections.

Copper type 2, or "normal" copper (Fig. 1A), is extensively found both in the laboratory and in nature. Most type 2 copper centers are three or four coordinated. One or more histidine ligands are always present in copper coordination: three or four imidazol rings are typically found in type 2 sites. Methionine, glutamate, glutamine, tyrosine or even exogenous ligands can complete the coordination sphere. Importantly, non-thiolate groups are coordinated. As a result of this coordination, copper type 2 UV–visible absorption spectra are characterized by low intensity d–d bands ($\varepsilon \leq 200\ M^{-1}\ cm^{-1}$), while their electron paramagnetic resonance (EPR) spectra display relatively high parallel coupling constants ($A_{||} \geq 140 \times 10^{-4}\ cm^{-1}$). This copper is located in multitude of enzymes [10,11] (superoxide dismutase, galactose oxidase, nitrite reductases, copper chaperones as the Atx1-like family, some ATPases…). Copper type 2 is also formed when copper binds to proteins or peptides with still unknown functions: the binding of copper to these proteins normally produces malfunctions and diseases [12], such as: the prion protein [13] (Kreutfeld Jakob or mad cows diseases), XIAP protein [14] (related to the Wilson disease), α-synuclein [15,16] (responsible for the Parkinson disease) or the Aβ-amyloid peptide [17] (Alzheimer's disease). All of them bind copper. This metal seems to accelerate or cause the aggregation of the polypeptide chain, with the subsequent formation of amyloidal

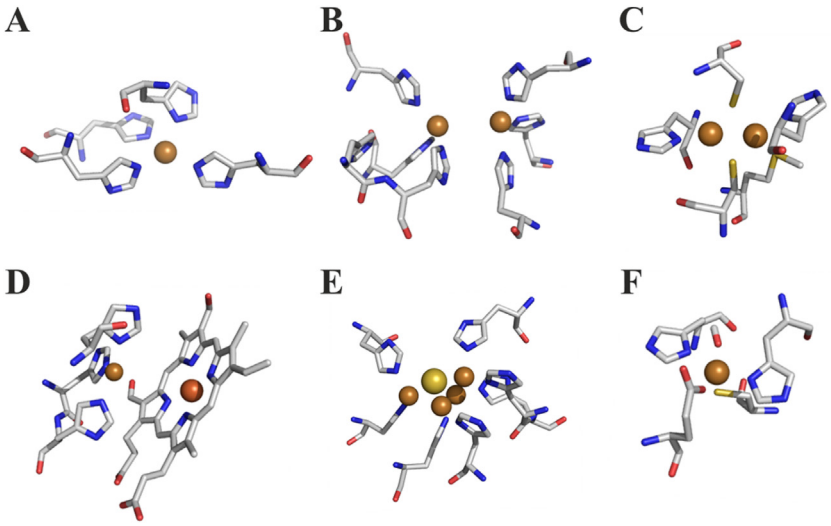

Fig. 1. Copper centers: A) Type 2, copper site of Cu/Zn superoxide dismutase from *Brucella abortus* (4L05 PDB code); B) Type 3, a tyrosinase from *Streptomyces castaneoglobisporus* (2ZMZ); C) Copper A, ba_3 cytochrome *c* oxidase from *Thermus thermophilus* (3S8F [155]); D) Copper B, cytochrome *c* oxidase (1V54 [156]); E) Copper Z, nitrous oxide reductase from *Paracoccus denitrificans* (1FWX [157]); F) Red copper, nitrosocyanin from *Nitrosomonas europaea* (1IBY [27]). See text for details. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)