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This Week's Citation Classic

Malkin R & Malmström B G. The state and function of copper in biological systems. *Advan. Enzymol. Relat. Areas Mol. Biol.* **33**:177-244, 1970. [Göteborg, Sweden]

The chemical properties of copper in proteins were correlated with biological function. Blue oxidases were shown to contain three types of copper. Nonblue Cu^{2+} (type 2) is spectroscopically normal, whereas blue Cu^{2+} (type 1) has unique properties owing to an asymmetric coordination forced upon it by the protein conformation. The third copper type is a metal pair which facilitates two-electron steps in dioxygen reduction. [The *SCI*® indicates that this paper has been cited in over 295 publications since 1970.]

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"Richard Malkin came from the University of California, Berkeley, in 1967 to work with me and Tore Vänngård in Göteborg during a two-year postdoctoral period. He participated in experimental work which caused a reorientation of our concepts concerning copper in proteins, particularly in the blue oxidases. This was the reason that we accepted an invitation in July 1968 from the editor of *Advances in Enzymology* to write a review on copper proteins, despite the fact that the proceedings[1] of a symposium on the subject had been published only two years earlier.

"Vänngård and I had published a paper[2] in 1960, in which we showed, with the aid of electron paramagnetic resonance (EPR), that laccase and ceruloplasmin contain Cu^{2+} in a unique coordination environment. We suggested that the unusual coordination is the result of the protein conformation, and that it also is responsible for the anomalously strong blue color of these proteins. Our report caused a storm of protest from inorganic chemists, who told us that only Cu^{1+} complexes could have such intense colors. Consequently, they argued, the same copper ion could not give both the EPR signal, which must stem from a Cu^{2+} ion, and the strong color. Our further experimental work,[3] however, showed that the original interpretation was correct, and Malkin and I discussed possible models which could explain the unique properties of blue or type 1 Cu^{2+} in our review.

"When Malkin started his work in our laboratory, it was generally believed that the blue oxidases do, in fact, also contain Cu^{1+}, as our group had shown[3] that only 50 percent of the total copper is detectable as Cu^{2+} by EPR. In 1968, I went to Rome to measure the kinetics of Cu^{2+} reduction in laccase, together with Eraldo Antonini, while Malkin stayed in Göteborg to study the thermodynamics of the same reaction, together with another postdoctoral fellow, James A. Fee. We exchanged experimental results by mail and initially expressed mutual skepticism about each other's findings. Eventually, we concluded that both sets of data showed that the EPR-undetectable ions are also present as Cu^{2+} , and we developed the model of an exchange-coupled Cu^{2+} -Cu^{2+} pair. The role of this pair in facilitating two-electron steps in the dioxygen reduction was discussed in the review, which was concluded with the suggestion that a similar mechanism may be operative in cytochrome c oxidase, the terminal respiratory enzyme in all aerobic cells. This hypothesis is now well proved by subsequent work.[4]

"There are, I think, several reasons why our review has been frequently cited. We provided the first comprehensive discussion of the classification of copper in proteins into three major types. More significantly is perhaps the explosive growth of bioinorganic chemistry during the 1970s. What first appalled the inorganic chemists became a great attraction, once they were convinced that blue proteins do indeed contain Cu^{2+} in a unique coordination environment. Blue copper has consequently become a favorite object of investigation for the bioinorganic chemist, as evidenced by a recent book."[5]

1. **Peisach J, Aisen P & Blumberg W E**, eds. *The biochemistry of copper.* New York: Academic Press, 1966. 588 p.
2. **Malmslröm B G & Vänngård T.** Electron spin resonance of copper proteins and some model complexes. *J. Mol. Biol.* **2**:118-24, 1960.
[The *SCI* indicates that this paper has been cited in over 175 publications since 1961.]
3. **Broman L, Mabmtröm B G, Aasa R & Vänngård T**. Quantitative electron spin resonance studies on native and denatured ceruloplasmin and laccase. *J. Mol. Biol.* **5**:301-10. 1962.
[The *SCI* indicates that this paper has been cited in over 90 publications since 1962.]
4. **Malmström B G**. Enzymology of oxygen. *Annu. Rev. Biochem.* **51**:21-59, 1982.
5. **Spiro T G,** ed. *Copper proteins.* New York: Wiley, 1981. 363 p.