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REVIEWS: CURRENT TOPICS

# The significance of copper chelators in clinical and experimental application

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**Abstract**

The essentiality and redox-activity of copper make it indispensable in the mammalian system. However, a comprehensive understanding of copper metabolism and function has not been achieved. Copper chelators have been used as an approach to provide insights into copper acquisition, distribution, and disposition at both the cellular and organism level. Unfortunately, the understanding of effective copper chelators is predominantly based upon their chemical structures and their reactions with copper. The understanding of the efficacy of copper chelators in the biological system has been equivocal, thereby leading to under- or misleading-utilization of these agents in clinical and experimental approaches. Current use of copper chelators in vivo almost exclusively either limits the availability or focuses on the removal of copper in mammalian organ system. There are at least two aspects of copper chelators that are yet to be explored. First, copper chelators preferentially bind either cuprous or cupric. As a result, they potentially modulate copper redox-activity without removing copper from the system. Second, copper chelators are characterized as either membrane-permeable or -impermeable, thus would serve as an organ-selective copper delivery or deprivation system to manipulate the biological function of copper. Here we review clinically relevant copper chelators that have been experimentally or clinically studied for their role in manipulation of copper metabolism and function, paying critical attention to potentially more valuable usage of these agents.


*Keywords:* Copper; Copper transport; Cuprous; Cupric; Chelator; Homeostasis

## 1. Introduction

The essentiality of copper ions in biological systems has long been recognized [1]. Copper ions are integrated components of some critical protein structures, involved in catalytic activities and crucial for regulatory functions. The average content of copper is only about 100 mg in human body, but there is virtually no free copper in the cell [2]. By coordinating to proteins and obtaining assistance from chemical ligands such as sulfur, oxygen and nitrogen, copper participates in mitochondrial respiratory reaction and energy generation, regulation of iron acquisition, oxygen transport, cellular stress response, antioxidant defense and several other important processes [1]. By regulating the activities of several critical copper-binding proteins such as cytochrome *c* oxidase (CcO), copper-zinc superoxide dismutase (Cu,Zn-SOD), dopamine β-hydroxylase (DBH), prion protein (PrP), tyrosinase, X-linked inhibitor of apoptosis protein (XIAP), lysyl oxidase, metallothionein (MT), ceruloplasmin and various others, copper exhibits its extensive role in living organisms from microbes to plants and humans [3].

In biological systems, copper ions usually exist in two oxidation states: cuprous ($Cu^{1+}$, reduced) and cupric ($Cu^{2+}$, oxidized). This redox activity has been utilized for catalysis by a number of enzymes [1]. Proteins take advantage of the redox nature of copper to achieve facile electron transfer reactions and gain activities [1]. However, the chemical properties that grant copper biologically useful are also potentially toxic. Redox reactions in which copper takes part generate hydroxyl radical and potentially result in severe damage to lipids, proteins, and DNA [4]. Moreover, the imbalance of copper homeostasis in humans causes serious health problems including neurodegenerative symptoms [5,6], cardiovascular structural and functional defects [7,8], bone metabolism disorders [9,10], musculature diseases [11,12] and deregulation of inflammatory responses [12,13].

Integrated approaches are required to understand the plethora of biological activities of copper in organisms. Copper chelators have long been the primary selection to advance the study of copper-related biological functions [14]. These chelators have proven to be valuable in clinical approaches to manipulate disease conditions due to alterations in copper metabolism [15]. Copper chelators have been principally used in three aspects: (1) the understanding of the molecular basis for copper and copper-binding proteins in biological system, (2) the treatment for diseases due to alterations in copper metabolism and (3) the diagnostic application for copper metabolic disorders.

Many compounds, observed with appropriate chelate denticity, suitable donor binding groups, and matching cavity size of geometric

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conformation, are suggested to form stable complexes with copper ions. However, copper ions in different oxidation state prefer different donor binding groups. $Cu^{1+}$ is the lowest oxidation state. It has a diamagnetic $d^{10}$ configuration and forms complexes with flexibility in geometric arrangements [16]. This means that $Cu^{1+}$ reasonably adopts tetrahedral, trigonal, or even linear geometries which are disfavored by other metals [16,17]. Chelate complexes of $Cu^{1+}$ are constructively prepared using relatively soft polarizable ligands comprising of thioethers, nitriles, cyanide, iodide and thiolates. $Cu^{2+}$ is the oxidized state of copper. It has a $d^9$ configuration, which favors amines, imines and oxygen donors to form square-planar, distorted square-planar, trigonal-pyramidal, and square-pyramidal geometric conformations [16]. Attributable to Jahn-Teller distortions, additional distorted octahedral may be observed as an axial elongation or a tetragonal compression in six-coordinate $Cu^{2+}$ complexes [17]. The geometry of the ligand field influences the redox state of copper. For example, ligands that impose a tetrahedral arrangement, explicitly unfavorable for $Cu^{2+}$ but reasonably favorable for $Cu^{1+}$, will destabilize the $Cu^{2+}$ form, shifting the reduction potential more positive in support of $Cu^{1+}$ [18,19]. The ligand-induced change in reduction potential allows to purposefully select a desired oxidation state in biochemical experiment. Numerous compounds were exploited as possible copper chelators and investigated for miscellaneous purposes. However, it is virtually an impossible mission to ascertain a perfect chelator for copper in any particular situation. Different chelators have different features that lead to their specific usage under certain circumstances.

The understanding above is based on the chemical structures of the compounds capable of chelating copper and the analysis of ligand-copper interactions. This understanding leads to clinical application of selected compounds for chelation therapy for copper overload or toxification. The goal is to remove excess copper from the organ system. However, an important factor, which has been not received equivalent attention in the design of clinical application of the chelate compounds, is the absence of free copper in mammalian cells [2]. The removal of $Cu^{1+}$ versus $Cu^{2+}$ from the organism leads to different consequences, which have not been fully understood or analyzed. On the other hand, the increase in total copper concentrations in the organism does not suggest an equal elevation of $Cu^{1+}$ and $Cu^{2+}$. It is exceedingly complicated that copper is coordinated to proteins, indicating the elevation of copper concentrations is associated with alterations in cellular protein composition and protein-protein interactions.

Pertaining to the above scenarios, the affinity of copper for different proteins varies and insinuates the possibility that a chelate compound may deprive copper from one protein but transfer it to another protein, thereby leading to alterations in copper intracellular trafficking and inter-organ transport by the chelate compounds. Therefore, there are at least two aspects of copper chelators that have not been fully explored in either clinical application or experimental studies: (1) copper chelators may change the balance between $Cu^{1+}$ and $Cu^{2+}$ in organisms, which may or may not be associated with changes in total copper concentrations, and (2) copper chelators may redirect the intracellular trafficking and the inter-organ transport. These applications require more comprehensive understanding of the biological aspects of copper chelators and copper speciation in the biological system.

In this review, we focus on clinically relevant and commonly used copper chelators, paying attention to the biochemical aspects of the structure and ligand analysis. Our efforts will be devoted to summarize the significant characteristics of typical chelating compounds, consisting of chemical name, structure, stability constants of copper and other common transition metals, complex solubility, and cell membrane permeability. The information presented in Table 1 is a general summary of the discussion. The following sections highlight some of the chelate agents widely used in clinical and experimental applications. The development of comprehension for these copper chelators in biological experiment and clinic application based on the analysis of the chemical mechanism of these compounds will be discussed in the following sections.

## 2. Copper-chelating compounds

### 2.1. Polyaminocarboxylate chelators

Polyaminocarboxylates are organic chelating agents consisting of a basic ligand of amino oxalic acid [$-N(CH_2COOH)_2$] (Fig. 1). There are two donor groups in these chelators: amino nitrogen and carboxyl oxygen. The former favors copper, zinc, mercury and cobalt, and the latter is able to chelate almost all metal ions in oxidation state. Currently, dozens of polyaminocarboxylate chelators have been applied in various fields.

Ethylenediaminetetraacetic acid (EDTA) is a classic chelator, commonly used in molecular biology and biochemical studies. It has a high affinity for $Cu^{2+}$ and the mechanism of transferring copper from chelation with peptides to EDTA has been studied as early as 1968 [14]. In the studies of physiological and pathological activity of copper-dependent compounds, EDTA was often used as a classic $Cu^{2+}$ chelator in experiments to modulate copper concentrations in the cell, or to terminate biochemical process of copper-containing compounds [21–23]. EDTA binds to copper and prevents copper-dependent biological events when it is added into media containing copper before the latter binds to targeted proteins or peptides. However, once the linkage between copper and protein has been formed, the addition of EDTA fails to despoil copper from the protein bound complex but impedes further cupro-protein formation [24,25].

Diethylenetriaminepentaacetic acid (DTPA) is another typical chelator with amino oxalic acid, comprising of octa denticities in its structure. The mechanism of action in copper chelation of DTPA is similar to EDTA. Both EDTA and DTPA are $Cu^{2+}$ chelators and both exhibit the ability to cross cell membrane. Without uncertainty, these two chelators are capable of inhibiting the Cu,Zn-SOD activity [26–28]. However, both EDTA and DTPA selectively inhibit nitric oxide (NO) transferase (S-nitrosoglutathione-reductase) activity of Cu,Zn-SOD but demonstrate no effect on the superoxide dismutase activity. It has been reported that EDTA or DTPA did not remove the bound copper in Cu,Zn-SOD, but EDTA or DTPA formed a complex with Cu,Zn-SOD in one chelator per homodimer [27]. Since the homodimer failed to bind two large ligands, a decreased access of large substrates such as glutathione and *s*-nitrosoglutathione at the catalytic site in the Cu,Zn-SOD-chelator complex was observed. Therefore, Cu,Zn-SOD may exhibit half-site reactivity, indicating both its free and chelator-bound forms possess the same SOD activity, but only the free form possesses s-nitrosoglutathione-reductase activity.

### 2.2. Acyclic amino chelators

The fundamental ligand of acyclic amino chelators is ethylene diamine [$-NHCH_2CH_2NH-$] (Fig. 2). Typical chelating agents in this class, for instance trientine and tetraethylenepentamine (TEPA), are homologues. Trientine includes four amino nitrogens in its chemical structure whereas TEPA includes five. Although the number of donor groups in trientine and TEPA is less than that in EDTA, their affinity for copper is significantly higher than EDTAs. The rationale for this preference resides in the greater stable formation of copper-chelate complex with a square-planar geometric conformation instead of other geometric conformations.

*N*,*N*,*N*′,*N*′-tetrakis (2-pyridyl-methyl)ethylenediamine (TPEN) is another chelator in this category. It is a tetrapyridylmethyl derivative