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Complexation, Structure, and Superoxide Dismutase Activity of the Imidazolate-Bridged Dinuclear Copper Moiety with β-Cyclodextrin and Its Guanidinium-Containing Derivative

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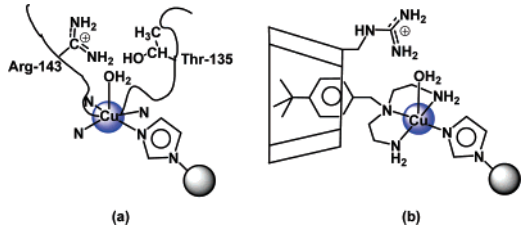
Cu,Zn−superoxide dismutase (Cu,Zn−SOD) is a ubiquitous enzyme with an essential role in antioxidant defense through catalysis of the disproportionation of $O_2^{\bullet-}$ under physiological conditions.[1−4] An imidazolate-bridged copper−zinc binding site, in which the copper ion has a distorted square pyramidal N_4O_w coordination sphere (one N atom from the bridging imidazolate), is absolutely essential for the SOD activity.[5−7] A recent study has shown that the misfolded Cu,Zn−SOD is associated with the inherited form of amyotrophic lateral sclerosis, a progressive degenerative disease of motor neurons.[8] To better understand structural factors on the origin of the highly efficient superoxide dismutation mechanism, a number of mimics for Cu,Zn−SOD have been reported.[9−17] From the pioneering work by Lippard et al.[10] to recent design of imidazolate-bridged Cu(II)−Zn(II) heterodinuclear complexes,[11−14] detailed studies on these mimics have provided valuable insights into the structure and function of the Cu,Cu−SOD and Cu,Zn−SOD active site.

Many studies on mutants of the enzyme have revealed that a few key charged residues in Cu,Zn−SOD, such as Arg-143 next to the copper(II) ion, promote electrostatic steering of the superoxide substrate toward the copper ion in the active site (Scheme 1a).[18−20]

Scheme 1. Schematic View of Cu,Zn−SOD Active Site (a) and Its Model (b)



Indeed, the mutation of Arg-143 to Lys leads to a decrease in the enzyme activity by 55%.[20b] However, some studies also showed that the diffusion-controlled rate of superoxide dismutation can be improved upon by increasing the charge density of the electrostatic patch.[21,22] It is still not clear whether the charged residues in Cu,Zn−SOD play a key role in dismutation of superoxide. Of those reported mimics, most models focus on the peculiarity of the imidazolate bridge, and almost no mimics of the electrostatic interaction of Arg-143 have been reported.[17] Since Cu,Cu−SOD shows slightly higher activity than Cu,Zn−SOD,[2] we have designed a new supramolecular model system through supramolecular inclusion of an imidazolate-bridged dinuclear complex with β-cyclodextrin (βCD) or mono-6-deoxy-6-guanidinocycloheptaamylose (βGCD) to mimic both the imidazolate-bridged active site and the Arg-143 (Scheme 1b).

The βGCD was synthesized according to previous literature.[23] A water-insoluble imidazolate-bridged dinuclear Cu(II) complex, $\{[Cu(L)(H_2O)]_2(im)\}(ClO_4)_3\cdot 2H_2O$ (**1**), where L is 4-(4′-*tert*-butyl)-benzyl-diethylenetriamine and im is imidazolate, was synthesized by mixing the ligand, Cu(II) ion, and imidazole in alkaline solution. Complex **1** was further assembled with βCD or βGCD, leading to the formation of supramolecular complexes, from which $\{[Cu(L)(H_2O)(\beta CD)]_2(im)\}(ClO_4)_3\cdot 21H_2O$ (**2**) was successfully isolated and structurally characterized by X-ray crystallography.[24] As shown in Figure 1, the two *tert*-butylbenzyl groups of the imidazolate-bridged dinuclear Cu(II) moiety respectively insert into cavities of the two βCD molecules along the primary hydroxyl side to form the supramolecular unit, $\{[Cu(L)(H_2O)(\beta CD)]_2(im)\}^{3+}$. Each Cu(II) ion has a distorted square pyramidal geometry with four nitrogen atoms and a H_2O molecule, which is very similar to N_4O_w, the coordination sphere of Cu(II) ion in the natural enzyme. Interestingly, the two βCD molecules form a head-to-head dimer though hydrogen-bonding of secondary hydroxyl groups (bond distances of 2.7−3.0 Å), resulting in a novel helical chiral chain.

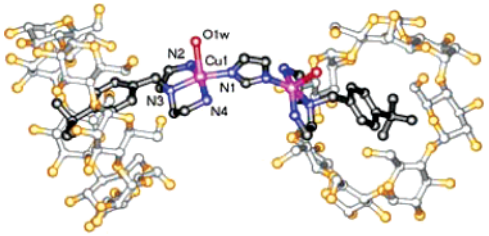


Figure 1. Supramolecular structure of $\{[Cu(L)(H_2O)(\beta CD)]_2(im)\}^{3+}$ cation in **2** at 173 K, showing the guest molecule inserted into βCD via hydrophobic interaction. All perchlorate anions and hydrogen atoms are omitted for clarity. Cu(1)−N(1) 1.978(4), Cu(1)−N(2) 1.951(6), Cu(1)−N(3) 2.014(7), Cu(1)−N(4) 2.011(11), Cu(1)−O(1W) 2.394(7), Cu···Cu 5.988 Å Color code: blue, nitrogen; black and metallic white, carbon; purple, copper; yellow and red, oxygen.

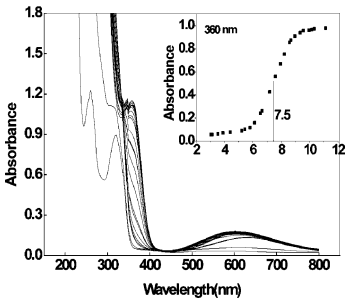


Figure 2. Changes of UV−vis absorption of **2** (1.0×10^{-5} M) as a function of pH (0.1 M $NaClO_4$, 25 ± 0.1 °C).

The changes in UV−vis absorption of **2** in aqueous solution were measured upon lowering the pH using $HClO_4$ (Figure 2). No changes in absorbance at 360 nm were evident at pH > 9 or pH < 5.5. From pH 9 to 5.5, the absorbance at 360 nm decreased significantly with the decrease in pH, from >90% to <15% of the

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Table 1. SOD Activities (IC_{50} Values) of Model Complexes (pH = 7.8)

complex	IC_{50} (μM)[a]
2	0.23
1/βGCD (1:2)[b]	0.20
1/βGCD (1:50)[b]	0.16
native SOD[c]	0.04

[a] The IC_{50} values for one Cu(II) ion. [b] Molar ratio. [c] Reference 12c.

original values. The curve has an associated pH value for half-dissociation of Cu(II) from **2** of 7.5 (pK_a). Since the pK_a value of $[Cu(L)(H_2O)(\beta CD)]^{2+}$ in the presence of βCD is 8.2, the slight increase in pK_a should not be due to the formation/dissociation of the dihydroxo species. Recent work showed that βCDs can prevent the formation of the μ-oxo dimer.[25] Therefore, the observed pK_a should correspond to the equilibrium between $\{[Cu(L)(H_2O)(\beta CD)]_2(im)\}^{3+}$ and $[Cu(L)(H_2O)(\beta CD)]^{2+}/[Cu(L)(H_2O)(imH)(\beta CD)]^{2+}$ species. For the self-assembly model complex using two independent mononuclear complexes and a free imidazolate ring, the imidazolate bridge is often unstable and readily breaks below pH ~9 in aqueous solution.[11,12] Although the imidazolate bridge in **2** has a similar structural feature, comparatively, its supramolecular chain through H-bonding strengthens the imidazolate bridge and results in its break equilibrium at physiological pH.

The temperature dependence of the magnetic susceptibility for **2** was examined in the range of 2−300 K (Supporting Information). The susceptibility data were fitted to the modified Bleaney−Bowers equation for exchange-coupled pairs of copper(II) ions,[26] and the following parameters were obtained: $g = 2.37$, $2J = -68.2$ cm^{-1}, and $zJ' = -4.50$ cm^{-1}. The obtained $2J$ value is slightly larger than that of Cu_2,Cu_2−SOD (-52.0 cm^{-1}) but is very close to those of reported binuclear (-76.2 cm^{-1}) and tetranuclear complexes (-70.0 cm^{-1}).[10e]

Although the supramolecular system of **1**/βGCD could not be characterized structurally, its SOD activity was investigated by NBT assay,[12c,27,28] together with **2** and the enzyme. The results can be duplicated within ±6%. Complex **2** exhibited a high SOD activity ($IC_{50} = 0.23$ μM), which is found only in complexes with highly stable imidazolate bridges.[12c,14] For a **1**/βGCD = 1:2 system, a slightly lower IC_{50} value (0.20 μM) was obtained, which showed that activity of **1**/βGCD presented was increased in comparison with that of **2**. To maintain enough $\{[Cu(L)(H_2O)(\beta GCD)]_2(im)\}^{5+}$ species in solution, the stoichiometric ratio of **1**/βGCD was further increased to 1:50, and lower IC_{50} value (0.16 μM) was determined. Under this condition, the guanidinium-containing **1**/βGCD system enhances its SOD activity at least by 30% in comparison with **2** in the absence of guanidinium (see Table 1 for a summary).

The catalytic function of the stable imidazolate bridge and the positive guanidinium next to the copper(II) ion can be understand according to the catalytic mechanism suggested by Bertini and Valentine et al (Supporting Information).[1a,2] Under the guide of the positive guanidinium, first, superoxide displaces a water molecule, binds directly to the copper ion, and gives up its electron. Second, the imidazolate bridge breaks and oxygen leaves. The N atom of the imidazolate gets its proton from bulk solvent. Third, superoxide binds again to the copper ion and accepts an electron. Finally, superoxide protons from a nearby water molecule and bridged imidazolate form hydrogen peroxide. The imidazolate bridge re-forms, and peroxide leaves under the guide of the positive guanidinium.

In conclusion, we have reported the preparation, structure, and SOD activity of a Cu-im-Cu/βCD supramolecular inclusion complex and its guanidinium-containing derivative. In this novel system, the independent imidazolate bridge can easily form and break at the physiological pH, and this facilitates proton transfer in the catalytic process. The positive guanidinium plays an important role for enhancing the SOD activity by guiding that superoxide to enter and peroxide to leave rapidly from the copper ion.

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Supporting Information Available: Synthesis, physical measurements, and X-ray crystallographic data (PDF, CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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