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Effect of the amide groups on superoxide dismutation catalyzed by copper(II) complexes of adamantane

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ABSTRACT

Four copper(II) complexes (**1**, $[Cu(L^1)(CH_3COO^-)](ClO_4)$; **2**, $[Cu(L^2)_2](ClO_4)_2$; **3**, $[Cu(L^2)_2Br]Br$ and **4**, $[Cu_2(L^2)_2(CH_3COO^-)_4]$; L^1 = *N*,*N*′-bis(2-pyridylmethyl)amantadine, L^2 = (pyridyl-2-amino)carbonyl adamantane) have been synthesized and characterized by elemental analysis, IR and single crystal X-ray diffraction. Copper ions in **1** and **2** have the distorted planar coordination configuration. The coordination geometry of **3** and **4** can be described as the distorted tetragonal pyramid. The adjacent two copper ions in **4** are bridged by four acetate anions. By using NBT assay at pH 7.8, the superoxide dismutase (SOD)-like activity (IC_{50}) of **1**–**4** were measured to be 0.21, 0.15, 0.22 and 0.90 μM, respectively. The enhancement in SOD-like activity of **2** suggests that the hydrogen bonds provided by the amide groups and the coordination configuration play an important role on the dismutation of $O_2^{\cdot-}$. Moreover, electrochemistry of complexes was determined by the cyclic voltammetry.

1. Introduction

During mammal metabolism, molecular oxygen can be converted into superoxide anion ($O_2^{\cdot-}$), which can further convert to the hydroxyl radical as a higher reactive oxygen species [1]. The hydroxyl radical nonspecifically oxidizes lipids, proteins, nucleic acids and sugars, resulting in various diseases including neurodegenerative, diabetes, inflammatory and carcinogenetic related processes [2]. Copper-zinc superoxide dismutase (Cu, Zn-SOD) is an essential enzyme in natural antioxidant system, which can catalyze the dismutation of superoxide to hydrogen peroxide and oxygen molecule under physiological conditions [3,4]. As shown in Scheme 1, a copper-zinc heterobimetallic complex is located at the active site of Cu, Zn-SOD, in which the copper(II) ion is coordinated by four histidine residues with a distorted square pyramidal coordination environment [5]. Due to the limitations of solution instability, immunogenicity, bell-shaped dose response curves, short half-lives, costs of production and proteolytic digestion, the direct utilization of Cu, Zn-SOD as pharmaceutical agents still exists many problems [6]. Therefore, the research of Cu, Zn-SOD mimics for the potential therapeutic applications are carried out towards obtaining stable, nontoxic and inexpensive low-molecular biomimetic molecules [7]. So far, various mono/di-copper(II) complexes as the model for the active site of Cu, Zn-SOD were reported [8,9]. However, the SOD-like activities of almost all mimics were relatively low, the highest SOD-like activity of reported mimics is about 40% of the bovine erythrocyte Cu, Zn-SOD [10–12]. Enzyme protein engineerings have revealed that the activities of Cu, Zn-SOD are correlated with coordination geometry of active site and the nearby amino acid residues which can provide weak interaction with the substrate [13]. Our previous work have constructed the supramolecular models with the beta-cyclodextrin and calixarene derivative [14,15], in which the synthetic routes are long and the assembly of host–guest compounds are challenging. Thus, the low molecular complexes containing the catalytic and collaborative groups could be feasible to mimic the active site of enzyme and the nearby weak interaction [16,17]. Di-(2-picolyl)amine and *N*-(pyridin-2-yl)formamide groups have been usually applied in the design of metalloenzyme models, due to the fascinating coordination property and bioactivity [18,19]. On the other hand, the adamantane derivatives exhibit the powerful therapeutic activity, because the hydrophobic adamantane backbones facilitate the drug molecules to penetrate the cell membrane [20,21]. For example, amantadine can effectively control the exuviating of influenza virus, restrain the virus infiltration into host cells by preventing influenza A2 and also alleviate Parkinson symptoms [22,23]. Therefore, the adamantane backbones were covalently linked with the

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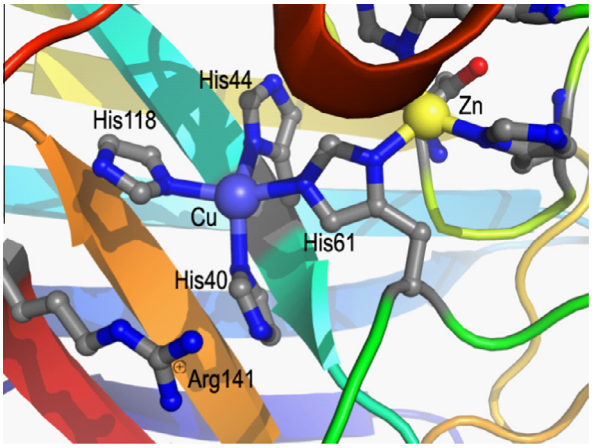
di-(2-picolyl)amine and *N*-(pyridin-2-yl)formamide groups to obtain the ligands. Furthermore, the copper(II) complexes were synthesized by the coordination interaction between the ligands and the different copper(II) salts (perchlorate, acetate and bromide), to explore the relationship between the superoxide dismutase-like activities and the structures of the copper complexes. Herein, we reported the synthesis and SOD activities of the four copper(II) complexes based on the adamantane derivatives (**1**, $[Cu(L^1)(CH_3COO^-)](ClO_4)$; **2**, $[Cu(L^2)_2](ClO_4)_2$; **3**, $[Cu(L^2)_2Br]Br$ and **4**, $[Cu_2(L^2)_2(CH_3COO^-)_4]$; where L^1 = *N,N'*-bis(2-pyridyl-methyl)-amantadine, L^2 = (pyridyl-2-amino)carbonyl adamantane). By using xanthine/xanthine oxidase-NBT assay at pH 7.8, the superoxide dismutase (SOD)-like activity (IC_{50}) of **1**–**4** were measured. Furthermore, DFT calculations were performed to elaborate the relationship between the complex structures and SOD activities. In addition, the properties of electrochemistry were determined by means of the cyclic voltammetry.

2. Experimental

2.1. Materials and instrumentation

Organic reagents were reagent grade and solvents used in this research were purified by standard procedures. Water used in all physical measurement experiments was double-distilled. The compound of *N,N'*-bis(2-pyridylmethyl)amantadine was synthesized from the reaction between halohydrocarbon and primary amine in the presence of potassium carbonate. The compound of (pyridyl-2-amino)carbonyl adamantane was synthesized with two steps from the 1-adamantanecarboxylic acid [24]. All compounds were confirmed by the elemental analysis and ^1H NMR spectra. Elemental analyses for C, H and N were obtained on a Vario EL III. As shown in Appendix A. supplementary material, infrared spectroscopy on KBr pellets was performed on Shimadzu IR Prestige-21 infrared spectrophotometer from 4000 to 400 cm^{-1}. The electronic spectra were measured on a Shimadzu UV-2450 spectrophotometer. The redox potentials of the complex were determined by CH Instrument 400c using the conventional three-electrode system of glass carbon electrode as the working electrode, platinum wire as the counter electrode, and Ag/AgCl electrode as the reference electrode. All the electrospray ionization mass spectrometry (ESI-MS) measurements were carried out by using a Finnigan LCQ DECA XP PLUS ion trap spectrometer operating in the positive ion mode and equipped with an orthogonal ESI source (Thermo Electron Corporation, USA).



Scheme 1. The structure of active site and nearby amino acid residue in Cu, Zn-SOD.

2.2. Synthesis of N,N'-bis(2-pyridylmethyl)amantadine (L^1)

To a solution of 1-amantadine hydrochloride (0.936 g, 5.0 mmol) and anhydrous potassium carbonate (2.762 g, 20.0 mmol) in 40 mL anhydrous acetonitrile was dropwisely added a solution of 2-(chloromethyl)pyridine hydrochloride (1.640 g, 10.00 mol) in 60 mL anhydrous acetonitrile with vigorous stirring. After heated to 353 K for 24 h under nitrogen, the reaction was cooled to room temperature, and then filtrated and evaporated to dryness under a reduced pressure. The residue was dissolved in methylene chloride (40 mL). Water (20 mL) was then added, the organic layer separated and dried with Na_2SO_4. After solvent evaporated, the residue recrystallized from ethanol/water to give white power product with 75% yield. *Anal.* Calc. for $C_{22}H_{27}N_3$: C, 79.24; N, 12.60; H, 8.16. Found: C, 79.12; N, 12.42; H, 8.32%. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.39 (s, 2H, H6-pyridine), 7.48 (m, 4H, H3,H5-pyridine), 7.01 (m, 2H, H4-pyridine), 4.02 (s, 4H, CH_2), 2.07 (d, 3H, CH-adamantyl), 1.79 (d, 6H, CH_2-adamantyl), 1.69 (m, 6H, CH_2-adamantyl). IR (KBr disc, cm^{-1}): 3438(s), 2903(vs), 2848(s), 1592(vs), 1568(m), 1477(m), 1359(m), 1342(m), 1307(m), 1125(m), 1101(m), 1083(m), 995(m), 943(m), 869(m), 765(s), 617(m).

2.3. Synthesis of (pyridyl-2-amino)carbonyl adamantane (L^2)

To a solution of 1-adamantanecarboxylic acid (1.802 g, 10.0 mmol) in 50 mL anhydrous toluene was dropwisely added thionyl chloride (60 mL) with vigorous stirring at 273 K. After heated to 343 K for 8 h under nitrogen, the reaction was cooled to room temperature, and then filtrated and evaporated to dryness under a reduced pressure. The residue was dissolved in 50 mL anhydrous toluene and then anhydrous potassium carbonate (2.762 g, 20.0 mmol) was added. Under ice-water bath, a solution of 2-(chloromethyl)pyridine hydrochloride (1.640 g, 10.0 mmol) in 60 mL anhydrous acetonitrile was dropwisely added with vigorous stirring. The reaction mixture was allowed to reach room temperature and stirred for an additional 3 h. Water (20 mL) was then added, the organic layer separated and dried with Na_2SO_4. After solvent evaporated, the residue recrystallized from ethyl acetate/ethyl ether (1:5) to give white power product with 68% yield. *Anal.* Calc. for $C_{16}H_{20}N_2O$: C, 74.97; N, 10.93; H, 7.86. Found: C, 75.12; N, 10.87; H, 7.68%. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.69 (bs, 1H, NH-amide), 8.35–8.38 (d, 1H, H6-pyridine), 8.241 (d, 1H, H3-pyridine), 7.78 (m, 1H, H4-pyridine), 7.08 (m, 1H, H5-pyridine), 2.11 (d, 3H, CH-adamantyl), 2.00 (d, 6H, CH_2-adamantyl), 1.76 (m, 6H, CH_2-adamantyl). IR (KBr disc, cm^{-1}): 3284(s), 3056(s), 2901(vs), 2846(s), 1666(s), 1528(s), 1438(s), 1291(m), 1232(m), 1169(m), 1079(m), 790(m).

2.4. Synthesis of $[Cu(L^1)(CH_3COO^-)](ClO_4)$ (**1**)

To 25 mL ethanol solution of L^1 (0.334 g, 1.0 mmol) was slowly added a solution of copper(II) acetate monohydrate (0.199 g, 1.0 mmol) in 15 mL ethanol with stirring. To facilitate the growth of crystal, sodium perchlorate (0.122 g, 1.0 mmol) was added. After the solution was stirred for 2 h at the room temperature and filtered, the solution was placed for slow evaporation. Ten days later, blue cubic crystals suitable for X-ray analysis were obtained with 45% yield. *Anal.* Calc. for $C_{24}H_{32}N_3O_7ClCu$: C, 50.26; N, 7.33; H, 5.65. Found: C, 50.04; N, 7.52; H, 5.95%. IR (KBr disc, cm^{-1}): 3567(s), 3385(s), 2909(m), 2848(w), 1612(s), 1574(vs), 1445(s), 1398(s), 1080(vs), 768(m), 624(s).

2.5. Synthesis of $[Cu(L^2)_2](ClO_4)_2$ (**2**)

To 25 mL ethanol solution of L^2 (0.256 g, 1.0 mmol) was slowly added a solution of $Cu(ClO_4)_2{\cdot}6H_2O$ (0.185 g, 0.5 mmol) in 25 mL