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RESEARCH PAPER

Synthesis of Co(II), Ni(II) Complexes Containing Aromatic Amines and Glycylglycine with Superoxide Dismutase-like Activity

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Abstract: Six metal complexes were synthesized by the reaction of Co(II), Ni(II) nitrate with the first ligand of Glycylglycine (Gly-gly) and the second ligand of 2,2'-bipyridine (2,2'-bpy)/4,4'-bipyridine (4,4'-bpy)/1,10-phenanthroline (Phen) in water solution (pH = 8.0–8.2). The compositions of these complexes were characterized by elemental analysis, thermal gravimetric analysis-differential thermal analysis (TG/DTA), infrared spectral (IR) method, and nuclear magnetic resonance (^{1}H NMR) method. The superoxide dismutase (SOD)-like activities and the interaction with human serum albumin (HSA) of these complexes were investigated. The results showed that all of the Co(II), Ni(II) complexes had the composition of [M(Gly-gly)(2,2'-bpy/Phen)(H_2O)]·2H_2O and [M_2(Gly-gly)$_2$(4,4'-bpy)(H_2O)$_4$]·4H_2O. Meanwhile, the IC_{50} of the complexes was about 0.327–0.564 μM that means the complexes have good SOD-like activities. The fluorescence spectra showed that these complexes could combine with HSA and be delivered by HSA molecules in human blood.

Key Words: Glycylglycine; Aromatic Amines; Complexes; Superoxide dismutase-like activity; Human serum albumin

1 Introduction

Superoxide dismutase (SOD) is a kind of metalloenzyme which can be widely found in animal, plant and microorganism. SOD plays an important functional role in cleaning the free radical in organisms. Natural SOD has some defects, such as great molecular weight, low stability, poor membrane permeability, easily decomposing by pepsin and high price, so its application is limited to a large extent. Therefore, the design and synthesis of some new compounds with high SOD activity that can reduce the defects of natural enzyme have been widely investigated in biochemistry[1–3]. Dipeptide, a kind of important biomolecule, has favorable physiological activity in organisms. Dipeptide as a ligand can effectively improve the bioavailability and the bioactivity, and reduce the toxic and side effects of complexes. The copper complex with dipeptide and aromatic amine was investigated as SOD mimics[4]. For example, Ding *et al*[5] synthesized the dipeptide-copper-polypyridine ternary complex with favorable SOD activity. Tabassum *et al*[6] synthesized the [Cu_2(Gly-gly)$_2$(ppz)(H_2O)$_4$]·2H_2O complex which by had oxidative cleavage to DNA molecules as well as excellent inhibiting effect to topoisomerase I. Meanwhile, the complex had low IC_{50} value of SOD activity, showing preferable application prospect. The researchers wondered whether the complexes of other transition metals with dipeptide had SOD activity or not. For this purpose, in this study, 6 noval complexes with Gly-gly and 2,2'-bipyridine (2,2'-bpy)/ 4,4'-bipyridine (4,4'-bpy)/1,10-phenanthroline (Phen) of cobalt(II) and nickel(II) were designed and synthesized. And nitrotetrazolium blue chloride (NBT) photoreduction was utilized to test their SOD activity as well as the influence of

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the concentration of complex on SOD activity. The results showed that the IC_{50} values of SOD activity of these 6 complexes were from 0.327 μM to 0.564 μM. Comparing with the IC_{50} value of natural SOD (0.0147 μM[7]) and the IC_{50} of similar complexes of copper reported before (0.467–0.522 μM[7] and 0.225–0.854 μM[8–12]), the synthesized complexes of cobalt and nickel had good SOD activity and superior effect in removing superoxide radicals. In addition, the SOD activity of complexes reduced with the increase of the molecular planarity of ligand. The matters with SOD activity would be transmitted by blood after entering the body. Human serum albumin (HSA) is the most abundant protein in blood circulation system, and it also plays a role in transporting and transferring endogenous and exogenous compounds. To explore the behavior of the synthesized complexes in blood, the interaction between these 6 complexes and HSA molecules was studied by spectroscopic method. The results indicated that the complexes could combine with HSA to form a new kind of compound. So the complexes could be conveyed by HSA molecules to remove the free radicals in body.

2 Experiments

2.1 Instruments and reagents

FTS-3000 spectrophotometer (Digilab Co. Ltd., United States) was used for infrared spectra test. The following instruments were used as well: TG/DTA-6300 Analyzer, a LS-55 fluorospectro photometer and a 2400-CHN elemental analyzer (all produced by PE company, United States) and a Mercury plus 400 superconducting nuclear magnetic resonance (Varian Medical Systems, Inc., United States).

Gly-gly ($C_4H_8N_2O_3$), a kind of biochemical reagent with purity of 98%, was purchased from Chengdu Enlai biological technology Co. Ltd., China. 2,2'-bpy, 4,4'-bpy, Phen and absolute ethanol were commercially available analytical pure. Methionine (MET) riboflavin (VB_2) and NBT were biochemical reagents obtained from Humei Chemical (China). Redistilled water was used in the whole experimental process.

2.2 Synthesis of complex

Aqueous solution (5 mL) containing 0.5 mmol of Gly-gly and 10 mL of absolute ethanol solution with 0.5 mmol of 2,2'-bpy were sequentially dropped into 5 mL of 0.1 M $Co(NO_3)_2$ solution. Then 1 M of NaOH solution was applied to adjust the pH value within the range of 8.0−8.2. With the protection of nitrogen, the mixture solution was stirred under refluxing at 80 ºC for 3 h and then stirred at room temperature for 8 h. Then it was filtered, and the filtrate was placed for standing and volatilization at room temperature. Several weeks later, some red granular crystals were dissolved out. After filtration, these crystals were dried and preserved. Other 5 kinds of complexes were synthesized in the similar way.

2.3 SOD activity test of complex

NBT photoreduction was adopted to measure the SOD activity of complex. The phosphate buffer solution (PBS) with pH 7.8 was used to prepare the mixed solutions containing the following matters: 33 μM of VB_2, 0.01 M of MET and 46 μM of NBT were as for blank group; the matters used by blank group and 0.6 μM of complex as for complexes group. Then the mixed solutions for the above groups were respectively placed in thermostatic water bath at 30 ºC for 10 min. After that, they were irradiated by fluorescent lamp for 3 min to measure the absorbance of the solution at 560 nm by using Type 722 spectrophotometer. The mean values of each mixed solution were obtained by parallel determinations for 3 times.

2.4 Interaction between complexes and HSA

Tris-HCl buffer solution with pH 7.15 was employed to prepare HSA solution with the concentration of 10 μM. Then the complex solutions above were added to react for 3 min at room temperature. The intervals of excitation wavelength and emission wavelength were fixed as $\Delta\lambda$ = 15 nm and $\Delta\lambda$ = 60 nm, respectively to scan synchronous fluorescence spectrum, the bandpass of entrance slit and exit slit was set as 5 nm, and the scanning speed was set at 240 nm min^{-1}.

2.5 Test of cytocompatibility of complex

The human hepatoma HepG2 cells were inoculated into a culture plate with 96 holes. Then 20 μL of 0.6 μM complexes solution was added respectively, while 1640 culture media with the same volume were added to the solution of control group. After 24 h, 10 μL of MTT solution was added. After incubation, a microplate reader was applied to measure their absorbance value (570 nm) to calculate the growth and survival rate of cells.

3 Results and discussion

3.1 Characterization of complex

3.1.1 Infrared spectra of complex

The infrared spectra (IR) of ligands and complexes from 500 cm^{-1} to 4000 cm^{-1} at room temperature were obtained by using KBr pellet (Fig.1 and Fig.2).

The amino vibration peaks of Gly-gly molecule appeared at 3288 cm^{-1} ($\nu_{as}(-NH_2)$) and 3061 cm^{-1} ($\nu_s(-NH_2)$), while the absorption vibration peaks of carboxyl were at 1705 cm^{-1} ($\nu_{as}(-COO^-)$) and 1308 cm^{-1} ($\nu_s(-COO^-)$). Moreover, the C=N vibration absorption peaks in aromatic amine occurred at