Synthesis and Unique Function of a Copper(II) Compound Possessing an Imidazole Moiety as an Anchor Group

Satoshi Nishino, Yuichi Sutoh, Takanari Togashi, and Yuzo Nishida

Department of Chemistry, Faculty of Science, Yamagata University, Yamagata 990-8560, Japan

Reprint requests to Prof. Y. Nishida. Fax:+81-23-628-4591. E-mail: yuzo@sci.kj.yamagata-u.ac.jp

Z. Naturforsch. **2007**, *62b,* 205 – 208; received September 11, 2006

A new copper(II) complex with a (bdpg-His) ligand was prepared and its structural properties were investigated both in crystalline and in solution states; (bdpg-His) = *N,N*-bis(2-pyridylmethyl)carnosine methylester. In the solid state, the complex has a dimeric structure, but in solution it partially dissociates into monomeric species, which have an imidazole moiety as an anchor group. This complex can prevent the precipitation of aggregated amyloid beta-peptide induced by zinc(II) ions, and affects the structure of the superoxide dismutase molecule, leading to the loss of the dimeric structure; these effects are probably due to the free imidazole group of the complex in solution.

Key words: Copper(II) Complex with a Histidine Anchor, Aggregation of Amyloid Peptide

Introduction

Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder and the most common cause of dementia among the elderly [1]. The clinical symptoms of AD include memory loss, particularly of recent events during the initial phases, and impairments in other cognitive domains that interfere with mood, reason, judgment and language. The "amyloid cascade theory", which has dominated AD research for the past 15 years, proposes that β-amyloid (Aβ) is a protein that spontaneously aggregates into amyloid fibrils which are somehow neurotoxic and cause dementia [2]. The rapidly growing knowledge of the metallochemical properties of Aβ, combined with advances in research of the neurobiology of metal-ion metabolism in the brain, have provided a model for AD.

At present, Zn^{2+} appears to be the major neurochemical factor responsible for aggregation of Aβ. Originally *in vitro* studies have shown that Zn^{2+} ions, at low micromolar concentrations, rapidly transform soluble Aβ into protease-resistant amyloid aggregates [3,4] and the formation of intermolecular His(Nτ)-Zn^{2+}-His(Nτ) bridges (Fig. 1) has been proposed [5,6]. Importantly, a rat and a mouse Aβ, which possess three amino acid substitutions that abolish the crucial bridging histidine-13, are not precipitated by Zn^{2+}, indicating that the presence of a histidine moiety is necessary for the aggregation of an amyloid peptide induced by zinc ions [6 – 8].

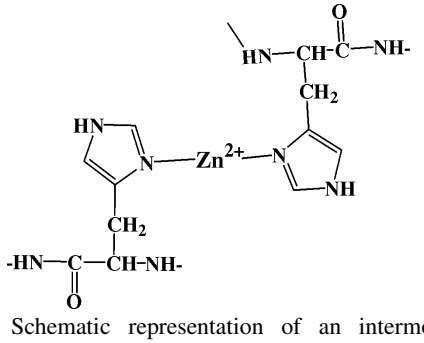


Fig. 1. Schematic representation of an intermolecular histidine-histidine bridge by zinc ions.

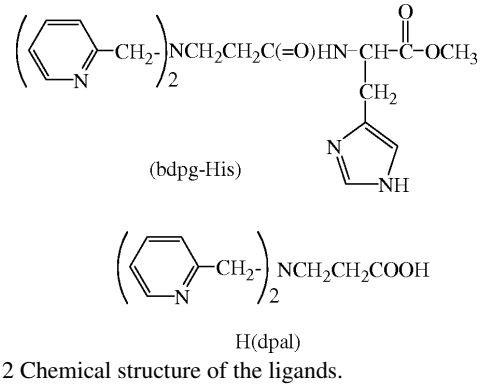


Fig. 2 Chemical structure of the ligands.

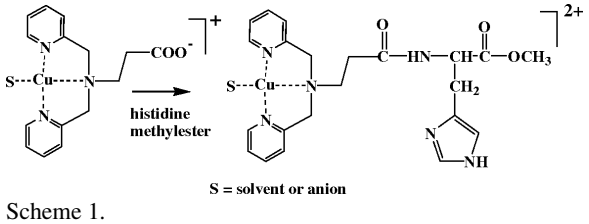
These facts are implying that the aggregation of Aβ in the presence of Zn^{2+} ions can be prevented by inhibiting the formation of the bridging structure. This may be accomplished by the introduction of another

histidine moiety into this region. For this purpose we have prepared a new copper(II) compound possessing a histidine moiety as an anchor group, $[Cu(bdpg\text{-}His)]^{2+}$ (for the structure of (bdpg-His), see Fig. 2), and confirmed that the precipitation of Aβ (1-40) by Zn^{2+} is greatly reduced by the addition of this copper(II) complex to the solution [9]. In this report, we show the preparation, the crystal structure, and the structural properties in solution of this copper(II) compound.

Results and Discussion

The role of the imidazole group of the $[Cu(bdpg\text{-}His)]^{2+}$ complex in solution

We obtained the $[Cu(bdpg\text{-}His)]Cl(PF_6)$ complex by the use of $Cu(dpal)ClO_4$ as a starting compound (Scheme 1). One might be able to synthesize the ligand (bdpg-His) from H(dpal) and histidine methylester, but it will be very difficult to get the pure target copper(II) compound by using the free ligand and copper(II) chloride, because coordination of the imidazole group toward the copper(II) ion may occur and the resulting product is not our target molecule.



Scheme 1.

As shown in Fig. 3, in the crystalline state two dications $[Cu(bdpg\text{-}His)]^{2+}$ form a dimeric structure in which the histidine moiety of one copper(II) chelate is coordinated to a second copper(II) ion. Each copper(II) ion is coordinated by four nitrogen atoms; two pyridine rings, an aliphatic amine and an imidazole of the histidine moiety form an almost square planar coordination geometry (see Table 1) around the Cu(II) center.

Both the CE and ESI mass spectra of the solution indicate that there are dimeric and monomeric species present in solution. In Fig. 4, the mass spectrum of the $[Cu(bdpg\text{-}His)]Cl(PF_6)$ (in water/methanol = 4/1) is shown. The peak at m/z = 242.5 corresponds to the dimeric species, $[Cu_2(bdpg\text{-}His)_2]^{4+}/4$. Another two peaks at m/z = 484.1 and 520.1 may correspond to $[Cu(bdpg\text{-}His)\text{–}H]^+$ and $[Cu(bdpg\text{-}His)Cl]^+$, respectively. These peaks suggest that the dimeric species present in the solid state partially dissociate in solution. This was also supported by the CE of the solution.

Table 1. Selected bond lengths (Å) and angles (°) of $Cu_2(bdpg\text{-}His)_2Cl_2(PF_6)_2$.

Cu1–N1	2.043(7)	Cu1–N2	2.007(8)
Cu1–N3	2.013(7)	Cu1–N12	1.982(7)
Cu2–N6	1.949(7)	Cu2–N7	2.069(7)
Cu2–N8	1.989(8)	Cu2–N9	2.029(7)
N1–Cu1–N2	83.0(3)	N1–Cu1–N3	82.7(3)
N1–Cu1–N12	177.6(3)	N2–Cu1–N3	165.7(3)
N2– Cu1–N12	97.9(3)	N3–Cu1–N12	96.3(3)
N6–Cu2–N7	178.0(3)	N6–Cu2–N8	99.1(3)
N6–Cu2–N9	96.8(3)	N–7Cu2–N8	82.6(3)
N7–Cu2–N9	81.6(3)	N8–Cu2–N9	164.1(3)

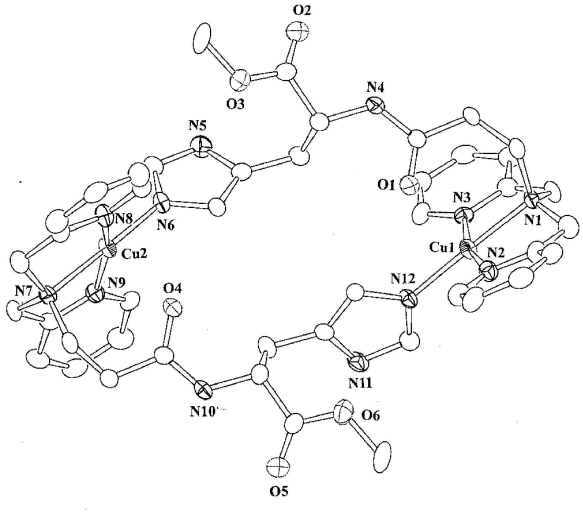


Fig. 3. Molecular structure of the tetracation in $Cu_2(bdpg\text{-}His)_2Cl_2(PF_6)_2$ in the solid state (ORTEP. H atoms omitted for clarity).

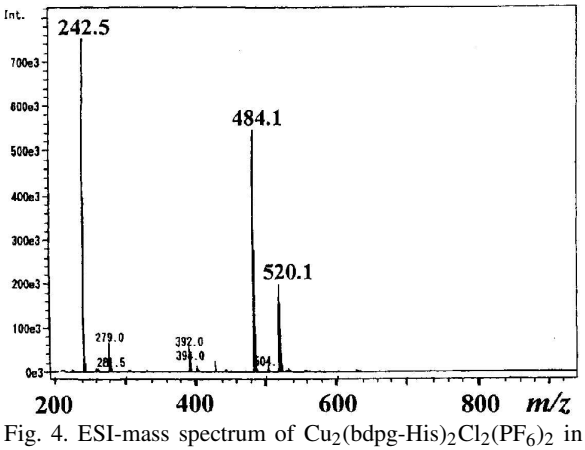


Fig. 4. ESI-mass spectrum of $Cu_2(bdpg\text{-}His)_2Cl_2(PF_6)_2$ in water/methanol (4 : 1) solution.

The CE of $[Cu(bdpg\text{-}His)]Cl(PF_6)$ shown in Fig. 5 is essentially the same as the one reported for Cu(dpaGMe) [11]; there are two peaks, a sharp one at 2.3 and

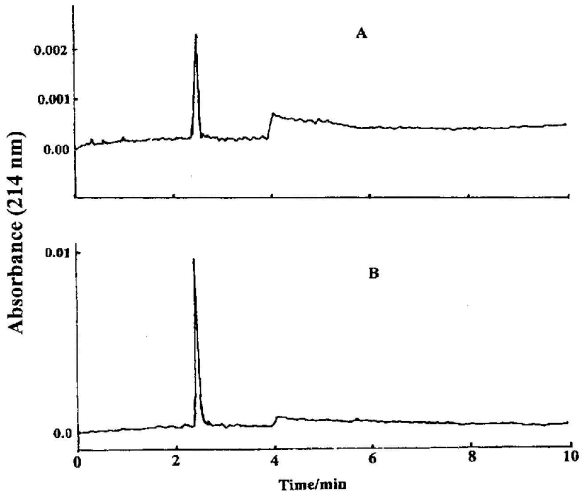
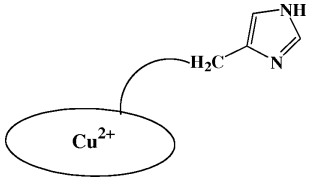


Fig. 5. Capillary electrophoregrams of Cu(bdpg-His)Cl(PF_6) (1 mM) in *tris*-buffer solution (10 mM, pH = 8.2). A: measured 20 min after the solution was prepared. B: measured 100 min after the solution was prepared.

a broad one around 4.1 $\sim$ 6 min, with the peak intensity observed at 2.3 min increasing gradually. Based on the previous data [9, 11], we conclude that the peaks correspond to the dimeric and monomeric species, respectively.



It seems quite likely that the imidazole group of the *monomeric* copper(II) complex functions as an anchor in solution as illustrated, and that the free imidazole moiety plays an important role in preventing the aggregation of amyloid β-peptide(1-40) by Zn^{2+} ions [9].

The [Cu(bdpg-His)]$^{2+}$ complex as a risk factor to induce "instability" of the dimeric SOD molecule

In Fig. 6, the CE profile of the SOD (superoxidedismutase; bovine Sigma) is shown (A). When the [Cu(bdpg-His)]$^{2+}$ complex solution was added to this solution, the peak height due to the SOD molecule greatly decreased as shown in (B), but the CE profile of the SOD molecule was not affected by the addition of a Cu(dpal) solution which has no free imidazole moiety. This clearly demonstrates that the Cu(bdpg-His) complex loosens the dimeric structure of SOD,

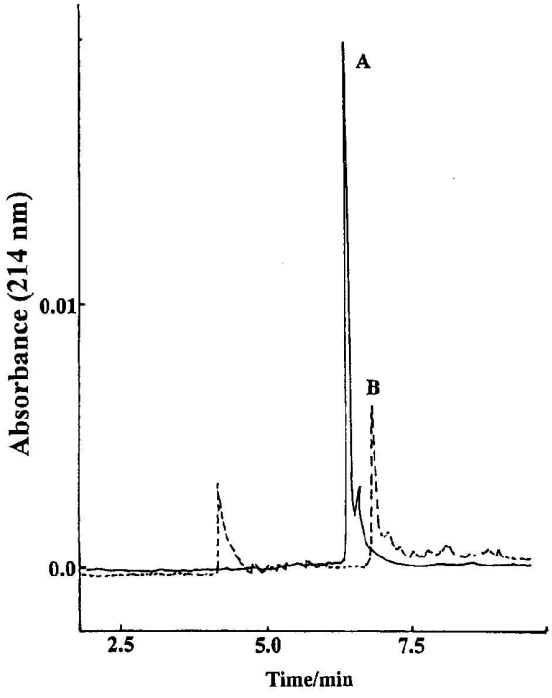


Fig. 6. Capillary electrophoregrams of (A) SOD (Sigma, bovine, 100 μL); (B) 10 μL of Cu(bdpg-His)Cl(PF_6) solution being added to solution (A).

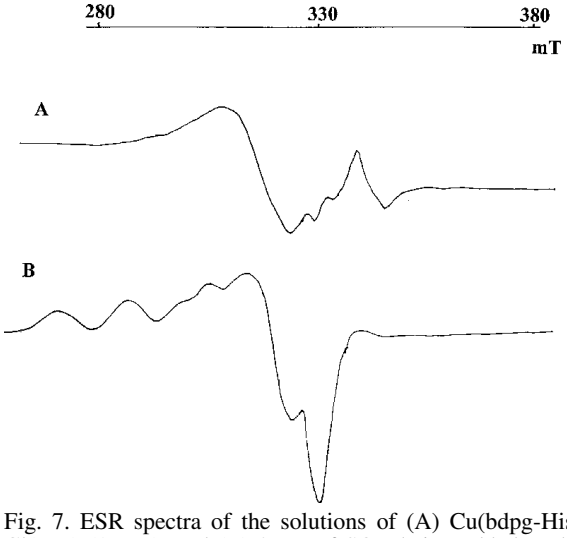


Fig. 7. ESR spectra of the solutions of (A) Cu(bdpg-His) Cl(PF_6) (1 mM), and (B) 2 mg of SOD being added to the solution (A) (1 mL). Under the conditions chosen, the ESR signals of copper(II) ions of SOD are negligible.

leading to partial dissociation into monomers [12], which may be due to the interaction between the free imidazole molecule and the surface molecules of the SOD. Fig. 7 shows the ESR spectra of the frozen

solutions (77 K) containing [Cu(bdpg-His)]Cl(PF_6). The ESR spectrum in Fig. 7(A) is characteristic for the dimeric (or aggregated) copper(II) species [13]. When SOD was added to the copper(II) solution, the ESR spectrum drastically changed to that characteristic for the isolated monomeric species, as illustrated in (B). These facts are implying that the dimeric structure of the Cu(bdpg-His) complex is destroyed by SOD, suggesting that the Cu(bdpg-His) complex can lead to an "instability" of the dimeric SOD molecule [14]. The presence of metal chelates such as $[Cu(bdpg\text{-}His)]^{2+}$ in the biological system may thus be a risk factor inducing sporadic ALS [15], in addition to hydrogen peroxide and several iron(III) chelates [12].

Experimental Section

Preparation of [Cu(bdpg-His)]Cl(PF_6)

To a dichloromethane solution (30 mL) containing Cu(dpal)ClO_4 (0.27 g, 1 mmol, for H(dpal) see Fig. 2) [10] and triethylamine (0.3 g, 3 mmol) histidine methylester dihydrochloride (Sigma, 0.24 g, 1 mmol) was added and the resulting solution was allowed to stand for 30 min at 273 K. A dichloromethane solution (10 mL) containing WSCD (420 mg: water-soluble carbodiimide, Peptide Institute Inc., No. 1020) was added dropwise to the above solution, and the solution was kept for 12 h at 273 K. The solvent was removed, and the residue was dissolved in methanol ($\sim$ 20 mL) containing NH_4PF_6 (500 mg, $\sim$ 3 mmol). After several days the precipitated blue crystals were filtered and recrystallized from a methanol-water solution. Yield 20 %. $CuC_{22}H_{26}N_6O_3ClPF_6{\cdot}4H_2O$ (738.5): calcd. C 35.78, H 4.64, N 11.38; found C 36.10, H 4.77, N 11.23.

Crystal structure determination of [Cu(bdpg-His)]Cl(PF_6)

Crystal structure data: T = 173 K, monoclinic, space group $P2_1$ (no. 4), a = 13.8588(9), b = 24.585(2), c = 8.8880(5) Å, β = 89.948(4)°, V = 3028.3(3) Å^3, Z = 2, D_{calc} = 1.530 g cm^{-3}; 6865 independent reflexions; $R1 = \Sigma||F_0| - |F_c||/\Sigma|F_0|$ = 0.078 for 6609 reflections with $I \geq 2.0\sigma(I)$. Selected bond lengths and angles are listed in Table 1. All calculations were performed using the TEXSAN crystallographic software package of Molecular Structure Corporation.

CCDC 200670 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge crystallographic Data Centre *via* http://www.ccdc.cam.ac.uk/data_request/cif.

Capillary electrophoregrams (CE): Capillary electrophoregrams of the $[Cu(bdpg\text{-}His)]^{2+}$ solutions were obtained with a CAPI-3200 (Otsuka Electronics Co.): zone electrophoresis, temperature 298 K; buffer solution 10 M, *tris*-HCl (pH = 8.2); 20 kV, uncoated column I. D. 75 μm, 50 cm; detection 214 nm. Copper(II) solution (10 μL of 1 mM solution in *tris*-buffer (10 mM, pH = 8.2)) was mixed with 20 μL *tris*-buffer (10 mM, pH = 8.2), and was eluted with *tris*-buffer solution (10 mM, pH = 8.2). The CE profiles of the solutions containing SOD were obtained with a Beckman/Coulter P/ACE MDQ: temperature 298 K; buffer solution, 10 mM *tris*-buffer (pH = 7.3); 20 kV, uncoated column I. D. 50 μm, 50 cm; detection 214 nm. Three solutions, 50 μL (SOD, 2 mg/1 mL), 50 μL (tris-buffer, pH = 7.3), and 10 μL of copper(II) complex (1 mg/1 mL solution) were mixed and eluted with the *tris*-buffer solution (pH = 7.3, 10 mM).

ESI-mass spectra: ESI-mass spectra (positive pattern) were obtained with an ESI-MS PE SCIEX API 300 for water/methanol (4 : 1) solutions containing a copper(II) compound at the Institute for Molecular Science (Okazaki, Japan).

[1] F. M. Laferla, S. Oddo, *Trends Molecular Medicine* **2005**, *11*, 170 – 176.
[2] A. I. Bush, *Trends Neurosciences* **2003**, *26*, 207 – 214.
[3] A. I. Bush, W. H. Pettingell Jr., M. D. Paradis, R. E. Tanzi, *J. Biol. Chem.* **1994**, *269*, 12152 – 12158.
[4] A. I. Bush, W. H. Pettingell Jr., G. Multhaup, M. D. Paradis, J. P. Vonsattel, J. F. Gusella, K. Beyreuther, C. L. Masters, R. E. Tanzi, *Science* **1994**, *265*, 1464 – 1467.
[5] T. Miura, K. Suzuki, N. Kohata, H. Takeuchi, *Biochemistry* **2000**, *39*, 7024 – 7031.
[6] C. C. Curtain, F. Ali, I. Volitakis, R. A. Cherny, R. S. Norton, K. Beyreuther, C. J. Barrow, C. L. Masters, A. I. Bush, K. J. Barnham, *J. Biol. Chem.* **2001**, *276*, 20466 – 20473.
[7] S. T. Liu, G. Howlett, C. J. Barrow, *Biochemistry* **1999**, *38*, 9373 – 9378.
[8] D. M. Morgan, J. Dong, J. Jaby, L. Kun, R. P. Apkarian, P. Thiyagarajan, D. G. Lynn, *J. Am. Chem. Soc.* **2002**, *124*, 12644 – 12645.
[9] Y. Sutoh, S. Nishino, Y. Nishida, *Chem. Lett.* **2005**, *34*, 140 – 141.
[10] T. Okuno, S. Ohba, Y. Nishida, *Polyhedron* **1997**, *16*, 3765 – 3774.
[11] A. Kishita, S. Nishino, Y. Nishida, *Synth. React. Inorg. Metal-Org. Nano-Metal Chem.* **2005**, *35*, 379 – 383.
[12] Y. Chiba, Y. Sutoh, Y. Nishida, *Z. Naturforsch.* **2006**, *61c*, 273 – 277.
[13] C.-L. O'Young, J. C. Dewan, H. R. Lilienthal, S. J. Lippard, *J. Am. Chem. Soc.* **1978**, *100*, 7291 – 7300.
[14] K. Yamanaka, D. W. Cleveland, *Neurology* **2005**, *65*, 1859 – 1860.
[15] Y. Nishida, *Med. Hypothesis Res.* **2004**, *1*, 227 – 245; *Annu. Rep.* CIN, **2005**, 2 – 32.