DFT studies on redox properties of copper-chelating cuprizone: Unusually high-valent copper(III) state

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ABSTRACT

We investigated the structures and redox properties of copper-chelating cuprizone using DFT-B3LYP calculations to gain insights into its toxicity causing neurodegeneration similar to lesions in prion diseases. We found that the copper-cuprizone complex has redox potentials of the Cu(III)/Cu(II) couple that range from -0.05 to $+0.98$ V (vs. normal hydrogen electrode), which are low enough to stabilize tri-valent copper(III) states. The resulting unusually high-valent copper(III) can be involved in cytotoxiciy to disrupt copper metabolism and/or to cause oxidative stress. This study on the redox behavior of copper-chelating cuprizone may provide not only an insight into its copper(III)-mediated toxicity but also a key to understanding for the pathogenesis of prion diseases.

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1. Introduction

Cuprizone (*N*,*N*′-di(cyclohexylidene)ethanedihydrazide) is a well-known copper chelator, which has been widely used as a copper-detecting agent [1], but has attracted much attention from biomedical researchers as a neurotoxic poison causing spongiosis in the brain similar to lesions in prion diseases [2–5].

Prion diseases are fatal neurodegenerative disorders, which are associated with conformational conversion of a normal cellular form of prion protein, PrP^C, into a misfolded or alternatively folded scrapie isoform, PrP^{Sc} [6,7]. According to the protein-only hypothesis, PrP^{Sc} itself is an infectious pathogen. However, there is controversy over the neurotoxic mechanism, when we assume that the causative agents, including PrP^C or PrP^{Sc}, are only composed of organic materials [8]. For example, a PrP-derived peptide fragment of PrP(106–126), covering the copper binding site around His111, is considered to be highly toxic, leading to a neurodegenerative process [9,10]. For this reason, pivotal neurotoxic roles of copper binding to this site have been discussed [11,12].

The neurotoxicity of cuprizone was originally found by Pattison and Jebbett [2–5]. In their pioneering works, the neurodegenerative-causing activity of cuprizone was found to occur under copper-chelating conditions. Recently, Messori et al. expected that a high-valent copper(III) state is stabilized by cuprizone [13]. Such the unusual oxidation state of copper(III) might be involved in the neurotoxic mechanism of cuprizone; however, the causal relationship has remained unresolved. The study on the copper(III)-

implicated redox behavior of cuprizone may provide a further insight into its neurotoxic mechanism and lead to an understanding of the copper-mediated pathogenesis of prion diseases.

In this study, we investigated the molecular structure and redox behavior of copper-chelating cuprizone using density functional theory (DFT) calculations, and intended to clarify its copper(III)-mediated neurotoxicity.

2. Models and methods

Cuprizone is a typical imine compound in which C=N double bonds are unstable during its metabolism. Nilsson suggested that the dihydrazone structure of cuprizone tends to hydrolyze partially, yielding the monohydrazone structure [1]. Messori et al. investigated the chemical composition of copper-cuprizone by elemental composition analysis, and identified to be $CuC_{16}H_{24}N_8O_4$ [13]. These results indicate that copper-chelating cuprizone had a chemical structure of $Cu(C_8H_{12}N_4O_2)_2$, which corresponds to a copper–cuprizone 1:2 complex, $Cu(CPZ)_2$, composed from a copper ion and two deprotonated monohydrazone units of cuprizone. However, up to now, no decisive structural evidence has been presented, such as using X-ray crystallographic analysis for copper–cuprizone.

We considered four models of $Cu(CPZ)_2$, shown in Fig. 1, which are discriminable by a set of coordination atoms and referred as (a) NNNN, (b) NONO, (c) ONON, or (d) OOOO, respectively. These copper–cuprizone complexes have isometric conformations in their cyclohexylideneamino group. The conformational difference in this part, however, may have no direct effect on the analysis of chemical reactivity of the complex, although analysis considering this

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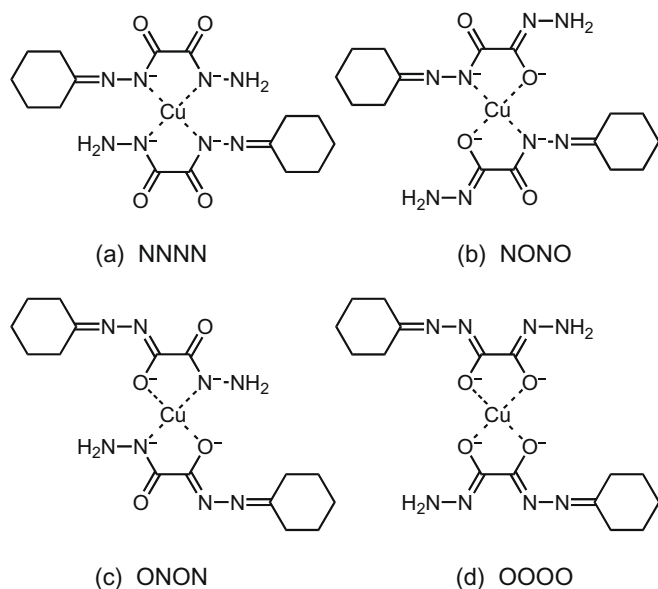
**Fig. 1.** Coordination modes of copper–cuprizone. Each has the chemical structure of $Cu(C_8H_{12}N_4O_2)_2$. In calculations, however, we used simplified structures of $Cu(C_5H_8N_4O_2)_2$ instead of the original to avoid unnecessary complicated problems.

difference is needed for the detailed study of the monomer itself. To avoid unnecessary complications, we used simplified models in which each cyclohexylideneamino group is replaced with an iso-propylideneamino group. The term $Cu(CPZ)_2$ will hereinafter be the abbreviation for the structure of $Cu(C_5H_8N_4O_2)_2$ instead of the original.

The redox (or electrode) potential provides a quantitative measure of reduction and oxidation reactions, where positive charges transfer spontaneously from an oxidizing agent having higher reduction potential toward a reducing agent having lower reduction potential. The direction of electrons flow is opposite. Here we note that in accord with the IUPAC convention [14,15], all redox potentials mentioned in this paper are to be intended as reduction potentials.

The standard hydrogen electrode (SHE) potential is defined to have a redox potential of exactly zero for the reference hydrogen electrode under standard conditions. The SHE potential is estimated from the energy change in the corresponding reactions, where one is a reduction reaction at the cathode, i.e.,

$$[Cu^{m+}(CPZ)_2]^{(4-m)-} + ne^- \rightarrow [Cu^{(m-n)+}(CPZ)_2]^{(4-m+n)-},$$
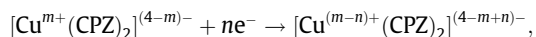
where n is the number of electrons transferred, and another is an oxidation reaction for the reference hydrogen electrode at the anode, i.e.,

$$n/2H_2 \rightarrow nH^+ + ne^-.$$
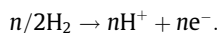
The SHE potential is given by

$$E_{SHE}^\circ = 1/nF(G^\circ[Cu^{m+}(CPZ)_2] - G^\circ[Cu^{(m-n)+}(CPZ)_2] - nG^\circ[H^+]$$
$$+ n/2G^\circ[H_2])$$
$$= E_{abs}^\circ[Cu^{m+}/Cu^{(m-n)+}] - nE_{abs}^\circ[H^+/H_2],$$

where G° denotes Gibbs free energy under the standard condition and F is the Faraday constant (i.e., elementary charge). Here absolute reduction potential of the hydrogen electrode, $E_{abs}^\circ[H^+/H_2]$, is assumed to be 4.36 V in aqueous solutions, which is now recommended as the preferred standard hydrogen electrode potential [16]. We calculated the absolute reduction potentials of $E_{abs}^\circ[Cu^{m+}/Cu^{(m-n)+}]$ using quantum chemistry methods with reference to several protocols used in previous studies [16–18].

Typical copper complexes are chemically stable in mono-valent or di-valent oxidation state under normal circumstances, whereas the higher tri-valent oxidation state is generally unstable. Messori et al., however, expected that cuprizone has potential to stabilize the unusually high-valent copper(III) state [13]. In calculations, thus, we considered three states of copper(I)-, copper(II)-, and copper(III)-cuprizone. For $Cu^{III}(CPZ)_2$, we considered both singlet ^1A and triplet ^3A spin states, respectively.

All calculations were performed using the GAUSSIAN 03 (revision D.01) package [19]. Geometry optimizations were performed at the DFT level using the B3LYP functional. The 6-311+G* basis set was used for copper, where the addition of the diffuse functions is needed to improve the estimation of redox properties [17]. The 6-31G** basis set was used for all hydrogen atoms. The 6-31G* basis set was used for carbon. The 6-31+G* basis set was used for nitrogen and oxygen, in which diffusion functions are needed for proper description of their anionic species.

Normal mode analyses were performed at the DFT-B3LYP level to characterize the optimized geometries as local minima, and to derive vibrational components in the partition function. The obtained harmonic partition functions were used to calculate contributions to the thermodynamical enthalpic and entropic corrections at standard temperature and pressure, i.e., 298.15 K and 1 atm, respectively, which are needed to calculate approximations of Gibbs free energy.

As previously reported [17], gas-phase calculation poorly models actual experimental redox properties. In this study, the solvation free energies were calculated in the presence of an implicit solvent, using the polarizable continuum model (PCM) [20]. The PCM model was configured to model water. In our approach, solvation energy was computed at optimized gas-phase geometry, since we found by recomputing the geometry under the implicit solvent that the degree of structural relaxation due to solvation effects is negligibly small.

3. Results and discussion

3.1. Copper-binding structure of cuprizone

Fig. 2 shows the structures of di-valent $Cu^{II}(CPZ)_2$ optimized at the DFT-B3LYP level. Each structure was confirmed to be locally stable by normal mode analysis. As shown in Fig. 2, a pair of cuprizone acts as tetradentate ligands for a central Cu^{2+} ion. Table 1 shows the distances of L_i-Cu dative bonds, where L means one of the coordination atoms, and subscript expresses the numbering that coincides with the set of indexes used in Fig. 2.

The primary coordination spheres of $Cu^{II}(CPZ)_2$ and $Cu^{III}(CPZ)_2$ had similar square planar arrangements; however, apparent differences in conformational parameters were recognized, as expected for the presence of copper in different oxidation and spin states. Table 1 shows that, as the formal oxidation state of copper decreases, the L_i-Cu dative bonds are elongated in the following order:

$$R[L_i - Cu^I] > R[L_i - Cu^{II}] > R[L_i - Cu^{III}(^3A)] > R[L_i - Cu^{III}(^1A)].$$

A similar relationship was found between oxidation states of copper compounds and ligand-metal distances by crystallographic analysis [21].

The striking difference in geometry characteristics between low- and high-spin states of $Cu^{III}(CPZ)_2$ is explained by the difference in their electronic configurations. The unpaired electron in the spin-triplet state of $Cu^{III}(CPZ)_2$ occupies an anti-bonding orbital that has a nodal plane cutting through the coordination bond, which corresponds to LUMO in the spin-singlet state. The L_i-Cu^{III} distances is, thus, longer in the spin-triplet ^3A state, whose MO includes an anti-bonding component, than in the spin-singlet ^1A state.