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The solution structure of the copper clioquinol complex

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ABSTRACT

Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline) recently has shown promising results in the treatment of Alzheimer's disease and in cancer therapy, both of which also are thought to be due to clioquinol's ability as a lipophilic copper chelator. Previously, clioquinol was used as an anti-fungal and anti-protozoal drug that was responsible for an epidemic of subacute myelo-optic neuropathy (SMON) in Japan during the 1960s, probably a myeloneuropathy arising from a clioquinol-induced copper deficiency. Previous X-ray absorption spectroscopy of solutions of copper chelates of clioquinol suggested unusual coordination chemistry. Here we use a combination of electron paramagnetic, UV–visible and X-ray absorption spectroscopies to provide clarification of the chelation chemistry between clioquinol and copper. We find that the solution structures for the copper complexes formed with stoichiometric and excess clioquinol are conventional 8-hydroxyquinolate chelates. Thus, the promise of clioquinol in new treatments for Alzheimer's disease and in cancer therapy is not likely to be due to any novel chelation chemistry, but rather due to other factors including the high lipophilicity of the free ligand and chelate complexes.

1. Introduction

Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline) (Fig. 1) is a lipophilic chelator of copper that was used extensively in the 1950s and 1960s as an anti-fungal and anti-protozoal drug [1,2]. Clioquinol's ability to chelate copper ions recently has triggered a surge of interest in it as a potential treatment of Alzheimer's disease [3–5] and in anti-cancer therapy [6,7]. In particular, the clioquinol-related compound known as PBT2 recently underwent clinical trials as an anti-Alzheimer's drug [8]. Alzheimer's disease is the sixth leading cause of death in North America, with an estimated cost of $203 billion dollars for 2013 in the United States alone [9]. Thus, the potential impact of clioquinol and related compounds is considerable.

Clioquinol originally was developed in 1934 as an external topical and gastro-intestinal antiseptic [1,2]. It was marketed under the drug name Entero-Vioform® as an over-the-counter remedy for traveler's diarrhea, and was believed to be completely safe as it was not thought to be absorbed from the gastro-intestinal tract. Aside from two isolated early reports of neurological problems [10,11], this unsubstantiated notion was backed up by nearly 35 years of apparently trouble-free use until clioquinol became suspected as the cause of an epidemic of subacute myelo-optic neuropathy (SMON) in Japan during the 1960s [12]. SMON patients show a variety of severe symptoms arising from degradation of the spinal cord, and optic and peripheral nerves. SMON sufferers also occasionally reported green urine and presented *lingua villosa verda* ("green hairy tongue"), both of which were subsequently shown to be due to an iron chelate of clioquinol [13,14]. Despite some controversy [15], clioquinol was withdrawn as a drug in the 1970s after convincing animal studies indicated that it could cause nerve damage similar to that observed in SMON [16]. The SMON epidemic ended following withdrawal, with clioquinol now accepted as the cause. It has long been known that copper deficiency can cause myeloneuropathy syndromes, typically associated with large intakes of zinc salts, which inhibit copper uptake. It has been suggested that the mechanism of action of clioquinol in causing SMON lies with its ability to chelate copper and that SMON was in fact a clioquinol-induced copper deficiency myeloneuropathy [17].

The structure of the Cu(II) clioquinol chelate previously has been investigated by X-ray crystallography [18] and in solution using X-ray absorption spectroscopy [7]. In the crystal structure the metal coordination is typical of Cu(II) in a square-planar type geometry with coordination by two clioquinols through phenolate and nitrogen donors with bond-lengths of 1.92 and 1.97 Å, respectively [18]. In marked contrast to this study, subsequent extended

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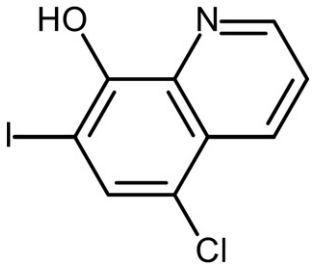

Fig. 1. Schematic structure of clioquinol (5-chloro-7-iodo-8-hydroxyquinoline).

X-ray absorption fine structure (EXAFS) analysis of the Cu(II) clioquinol complex concluded that in solution the metal was octahedrally bound by six similar donors (oxygen or nitrogen) with a bond-length of 2.36 Å [7]. These authors also concluded that the oxidation state of the copper had been reduced from its initial Cu(II) level [7], whereas the crystal structure indicated Cu(II) with a typical square planar type coordination [18]. The two studies also differed in that the crystallographic samples were prepared using a 2:1 clioquinol:Cu ratio giving two coordinating clioquinols [18], whereas the EXAFS study employed equimolar copper to clioquinol ratios in solution [7].

In order to address this discrepancy and to further characterize clioquinol due to its therapeutic potential for Alzheimer's disease and cancer, we present herein an X-ray absorption spectroscopic study of the complexes of copper with clioquinol. We show that the solution structures are tetragonally distorted species more typical of Cu(II) and closely related to the structure observed crystallographically.

2. Materials and methods

2.1. Samples

Dimethyl sulfoxide (DMSO) solutions of anhydrous $CuCl_2$ and clioquinol were mixed to a final concentration of 5 mM Cu for both stoichiometric (5 mM final) and excess (20 mM final) clioquinol. Solutions were loaded into acrylic sample cuvettes with a 1 mm X-ray path length, frozen in liquid nitrogen and stored at liquid nitrogen temperatures prior to spectroscopic measurements. The 1 mm X-ray path length is smaller than the usual condition for copper solutions (2–3 mm) and was selected because the high X-ray cross-section of DMSO relative to other common solvents comprised of lighter atoms (e.g. water, ethanol, etc.) meant that longer path length samples were essentially X-ray opaque. Samples for electron paramagnetic resonance (EPR) spectroscopy were prepared using 99.90 % enriched ^{63}CuO, obtained from Isoflex USA (San Francisco, CA, USA), converted to $CuCl_2$ with HCl, in DMSO solution at 1 mM final copper concentration and frozen in 3 mm external diameter quartz tubes (Wilmad-LabGlass, Vineland, NJ, USA).

2.2. EPR spectroscopy and UV–visible spectroscopy

Cu(II) EPR spectra were recorded on a JEOL RE1X spectrometer (JEOL USA, Inc., Peabody, MA, USA) at the Stanford Synchrotron Radiation Lightsource. Spectra were recorded at 100 K with 0.1 mT modulation amplitude and were integrated by reference to a 1 mM aqueous Cu(II)–EDTA standard,with field calibration using a diphenylpicrylhydrazyl standard. UV–visible spectra were recorded using a Cary 50 instrument (Agilent, Santa Clara, CA, USA).

2.3. X-ray absorption spectroscopy data collection

X-ray absorption spectroscopy (XAS) measurements were conducted at the Stanford Synchrotron Radiation Lightsource with the SPEAR storage ring containing 500 mA at 3.0 GeV, using the data acquisition program XAS Collect [19]. Copper K-edge data were collected on the structural molecular biology XAS beamline 7-3, employing a Si(220) double-crystal monochromator. Beamline 7-3 is equipped with a rhodium-coated vertically collimating mirror upstream of the monochromator with harmonic rejection accomplished by setting the mirror cutoff angle to 12 keV. Incident and transmitted X-ray intensities were monitored using nitrogen-filled ionization chambers with a sweeping voltage of 1.6 kV. X-ray absorption was measured as the Cu K_α fluorescence excitation spectrum using a germanium array detector (Canberra Ltd. Meriden, CT, USA) [20] and employing nickel filters with a Soller slit assembly to maintain germanium detector count-rates within the pseudo-linear regime [20]. During data collection, samples were maintained at a temperature of approximately 10 K using an Oxford Instruments liquid helium flow cryostat. Each data set was an accumulation of between four and ten scans each of 35 min duration. The near-edge portions of successive scans were examined for characteristic progressive changes that are indicative of photoreduction [21,22] and these were not observed under the conditions of our experiments. The energy was calibrated by reference to the absorption of a copper metal foil measured simultaneously with each scan, assuming a lowest energy inflection point of 8980.3 eV. The energy threshold of the extended X-ray absorption fine structure (EXAFS) oscillations ($k = 0$ Å^{-1}) was assumed to be 9000.0 eV. Data were truncated at $k = 13.7$ Å^{-1} due to noise. No significant contamination from traces of Zn, which would arise at $k \approx 13$ Å^{-1}, was apparent in any of our data.

2.4. XAS data analysis

The EXAFS oscillations $\chi(k)$ were quantitatively analyzed by curve-fitting using the EXAFSPAK suite of computer programs [23] as previously described [24,25], using ab initio theoretical phase and amplitude functions calculated using the program FEFF version 8.25 [26,27]. No smoothing, filtering or related operations were performed on the data.

2.5. Density functional methods

Density functional theory (DFT) calculations employed the programs Dmol^3 Materials Studio Version 6.1 [28], or Gaussian 09, revision C.01 [29]. Dmol^3 calculations used Becke exchange [30] and Perdew correlation [31] functionals both for the potential during the self-consistent field procedure, and for the energy. Double numerical basis sets included polarization functions for all atoms. Calculations were spin-unrestricted and with DMol^3 all-electron core potentials were used, whereas Gaussian calculations implemented an effective core potential for the Cu-atom. Gaussian calculations were performed without symmetry constraints (except in the case of the C_{2h} symmetry complex) using the B3LYP hybrid functional method [29]. A mixed basis set approach was employed for geometry optimizations and subsequent harmonic frequency calculations, using the LANL2DZ basis set [32,33] for the Cu, Cl and I atoms and the 6-311+G(d,p) basis set for C, O, N, S and H-atoms. Structures were considered optimized when the change in energy between subsequent optimization steps fell below 0.03 J mol^{-1}.

3. Results and discussion

Although clioquinol in isolation is slightly water soluble, complexes of clioquinol with Cu(II) are nearly completely insoluble in water. As a result of this low solubility we were unable to study the species in an aqueous environment; indeed nearly all experimental work reported to date on such complexes has used DMSO as solvent.

Fig. 2 compares the X-ray absorption near-edge spectra of the copper clioquinol complexes for stoichiometric and excess clioquinol. The spectra are quite distinct. Both show a small 1s → 3d transition close to 8979 eV, although for the complex formed with excess clioquinol this transition shows a somewhat higher peak energy of 8979.4 eV compared with 8978.7 eV for the stoichiometric complex. The presence