

Syntheses, Characterizations of Copper (II) with Amine Coupled Naphthoquinone Complex as Antioxidant

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Abstract

The presented study investigates application of Cu(II) complex (Cu-1) with Amine coupled to Naphthoquinone as Antioxidant agent. Cu-1 complex is synthesized with ligand L- 1 formed by amine coupling at C-2 positions of naphthoquinones. Cu-1 performs valence tautomeric distorted octahedral coordination established by IR, EPR, magnetic susceptibility measurements, and CV studies. Cu-1 also acts as functional model of SOD confirmed by DPPH method. Cu-1 with L-1 is tested for antioxidant activities. Their antioxidant activities are screened on DPPH assay. Both activities are compared & correlated with structures and e- transfer routes.

Keywords: Lawsone; SOD; DPPH; Synthesis copper complexes.

Introduction

Superoxide anion is essential for the biological defense system against the invasion of bacteria and viruses. On the other hand, the superoxide anion has been known to be important in the pathogenesis of many disease processes, including inflammatory damage, membrane and DNA damage, ageing and ultimate cancer can be formed [1,2]. SOD is found abundantly in many organisms, from microorganisms to plants and animals, since superoxide radicals are toxic to living cells, oxidizing and degrading biologically important molecules, such as lipids and proteins. Since the active centre of metalloenzymes resembles a simple metal complex, the preparation of complexes with similar structure is a promising way of mimicking the enzymes.

Structural and/or functional modelling the active sites are usually done by preparing complexes having the appropriate metal ion(s) and ligands of the same or similar structures found in the enzyme. The aim of this work was to prepare enzyme mimicking metal complexes and test them in various enzymatic reactions. Quinones are known for their pharmacologic properties [3] and for taking part in many biological processes, such as respiration and photosynthesis. Due to their binding ability, the coordination of these chelating compounds to transition metal ions has also been the subject of growing interest. The main motivations for the synthesis of novel naphthoquinone metal complexes are the study of their biological applications and the investigation of their magnetic properties. Ligands which contain potentially bridging phenoxo oxygen and nitrogen donor atoms have been widely used in the synthesis of copper complexes [4]. As the result of our ongoing investigations of biologically active 2-(*o*-Hydroxy Anilino)-1,4-Naphthoquinones and of their copper(II) complexes.

Experimental

Synthesis and characterization(L-1)

The solvents, methanol were purified according to the procedure cited in the literature [5]. L-1 was synthesized by adding solution of 1 mmole of 2-hydroxy-1,4-naphthoquinone (0.174 g) dissolved in 25 ml anhydrous methanol to the stirring solution of 1 mmole of 2-aminophenol (0.109 g) dissolved in 25 ml anhydrous methanol. The solution mixture was stirred and refluxes for 6-8h until it become dark Orange. The separated solid thus obtained was washed with anhydrous methanol and dried in vacuum. mp 190° C Microanalysis required for C,72.20; H,4.19; N,5.30 Calc.for C₁₆H₁₁NO₃ C,72.38;H,4.15;N,5.27.

Synthesis of compound Cu(L-1)₂

All chemicals used in the synthesis were obtained from Sigma Aldrich Chemical Co. To a solution of CuCl₂ .2H₂O 1 Mm(0.170) in 25 ml anhydrous methanol, a solution of L-1, 1 mM(0.530) in 25 ml of methanol, was mixed with constant stirring. This mixture was stirred for 72 h and reflux for 6-8h. A Brown colored precipitate, [Cu (L-1)₂]. m.p: 196-198° C; Anal. Calcd. for C₃₂H₂₀N₂O₆Cu C,64.64;H,3.36;N,4.71 Found :C,64.32;H,3.18;N,4.24.

Spectral characterization

Carbon, hydrogen and nitrogen were determined using CHNS analyzer on FLASH EA 1112 series (Thermo Electron Corporation) at Chemistry Department, Payame Noor University, 19395-4697 Tehran, Iran. Voltammetry employed a conventional three-electrode system, by using either a PGSTAT101 and glassy carbon working electrode. The scan rate for cyclic voltammetry results reported was 100 mV s⁻¹.

Cyclic voltammogram (CV) was recorded on an electrochemical analyser (PGSTAT101). The three-electrode cell includes a working electrode as a platinum or glassy carbon referenced with an Ag/AgCl electrode, a platinum wire as an auxiliary electrode. About 0.1 M tetraethyl ammonium perchlorate (TEAP) was used as a supporting electrolyte. Purified nitrogen atmosphere was maintained in the cell during CV run.

Estimation of metal ion in the complexes was done on Atomic Absorption Spectrophotometer using Perkin Elmer-3100 model at Chemistry Department, Payame Noor University, 19395-4697 Tehran, Iran. Estimation of metal ion from complex was done using Kjeldahl flask decomposition method [6]. Infrared spectra of copper complex were recorded as KBr pellets on FTIR-8400 Shimadzu infrared spectrophotometer at Chemistry Department, Payame Noor University, 19395-4697 Tehran, Iran. The X-band EPR spectra of complex was recorded on BRÜKER EMX EPR spectrometer at NCL, Pune-7 operating at X-band (9.5 GHz) and 100 kHz field-modulation. The electronic spectra of ligand and metal complex in spectra grade dimethyl sulphoxide (DMSO) were recorded on UV-2550, Shimadzu UV-Visible spectrophotometer at Chemistry Department, Payame Noor University, 19395-4697 Tehran, Iran.

Result and Discussion

Spectral characterization and cyclic voltammetry studies

The experimental spectrum of L-1 shows IR absorptions as follows: $\nu_{\max} / \text{cm}^{-1}$ 3280 (s)(NH), 3060 (sbr)(OH), 2700 (mbr) and 2623 (mbr)(O - H..O)(C - H..O), 1681(m) (C= O), 1544 (s) (N - H..O). The experimental spectrum of Cu-1 shows IR absorptions as follows: $\nu_{\max} / \text{cm}^{-1}$ 3280(s)(NH), 3060 (mbr)(OH), 3062 (w) and 3082, 565(s) (Cu-N), 923(w) (Cu-O).

Among copper dependent oxidases there are two specific enzymes GOs and CAOs in which Cu(II) is associated with redox active organic cofactor Tyr / TPQ[7-9] respectively. Since mononuclear CAOs cycles between Cu(II)/ Cu(I) oxidation states Cu(III) redox state is not biologically relevant [7]. Recently functional modelling with the help of cyclic voltammetry technique is reported by Tatiana and coworkers [10], using Cu(I) complexes of 2,2'-biquinolyl and 2,2'-quinolyl pyridine polymer ligands. While Kaim et al[11] have reported redox isomerism in mixed ligand copper complexes as model compounds of CAOs, having Q/ SQ redox potentials $E_{1/2}$ in range of -0.83V to -1.21V performing Cu(I)/ Semiquinone vs Cu(II)/ Catecholate forms. With this background we analyse redox reactions in Cu-1 to Cu-6 complexes.

Cyclic voltammograms (CVs) of Cu-1 complex has been recorded in DMSO solution containing 0.1M NEt_4ClO_4 at 298K, all potentials quoted refer to measurements run at a scan rate(ν), of 100mV.s⁻¹ and the cyclic voltammograms results are presented in Table1.

Table 1: CV data of Cu-1.

E _{pc} =-0.259V E _{pa} =-0.40V	$\Delta E=0.141V$, $(E_{1/2})_1 = 0.329 V$ (4HNQ-N-CAT)(4HNSQ-N-Q) \rightleftharpoons (4HNSQ-N-CAT)(4HNSQ-N-SQ)
E _{pc} =-0.564V E _{pa} =-0.68V	$\Delta E=0.116V \sim 2e^-$, $(E_{1/2})_2 = -0.622V$ (4HNSQ-N-CAT)(4HNSQ-N-SQ) \rightleftharpoons (CAT-N-CAT)(4HNSQ-N-CAT)
E _{pc} =-1.01V, E _{pa} =- 1.065V,	$\Delta E=0.055V \sim 1e^-$, $(E_{1/2})_3 = -1.0375V$ (CAT-N-CAT)(4HNSQ-N-CAT) \rightleftharpoons (CAT-N-CAT) ₂

The electrochemical properties are analyzed according to Pierpont's Schiff Base Biquinone complexes[12] which are analogues to our complex system.

CV of Cu-1 exhibits, two, 2e⁻ transfer and one, 1e⁻ transfer reversible processes necessarily ligand based, occurring at negative half wave potentials such as (E_{1/2})₁ = -0.329V (E_{pc} = -0.259V, E_{pa} = -0.40V, $\Delta E = 0.141V \sim 2e^-$), (E_{1/2})₂ = -0.622V (E_{pc} = -0.564V, E_{pa} = -0.68V, $\Delta E = 0.116V \sim 2e^-$), (E_{1/2})₃ = -1.0375V (E_{pc} = -1.01V, E_{pa} = -1.065V, $\Delta E = 0.055V \sim 1e^-$). These can be assigned to the couples.

The stepwise assignments of redox reactions particularly (E_{1/2})₁ and (E_{1/2})₃ are analogues to radically coordinated Cu(PPh₃)₂ (PhenoxSQ) complex reported by Pierpont.

E_{pc3} and E_{pc2} peaks possess large current due to high concentration of NSQ/SQ species, the difference between (E_{pc3}-E_{pc2}) = 0.244V is corresponding to 24.02kJ/mol electrochemical energy of radical form. This energy is nearly matching with energy of activation for evolution of coordinated radical ligand (E_a = 26.95kJ/mol) in Cu-1 as estimated from its pyrolysis.

As the solvation energies play the significant role in charge transfer mechanism in redox active ligands. Solvation energies can be estimated from thermal and electrochemical energies such as: $FE^{\circ}_i = I_i - \Delta_i G^{\circ}_s + T \Delta_i S^{\circ}_c + \text{constant}$ where I_i , is the gas phase adiabatic ionization potential of reduced form in complex.

I_i can be replaced by $I'_i - \Delta H_c$ where I'_i phase ionization potential of the free ligand i.e. E_a (energy of activation) from TG data. ΔH_c represent the difference is stabilization of ligand in reduced or oxidized form of complex in the gas phase. Hence for the ligand, equation can be modified as, $FE^{\circ}_i = I'_i - \Delta_i G^{\circ}_s + T \Delta_i S^{\circ}_L + \text{constant}$ $\Delta_i G^{\circ}_s$ represents difference of the real free energy of solvation between the reduced and oxidized form. Last two terms can be eliminated or approximated by taking difference in energies and finally to evaluate solvation energies, $F\Delta_{ij}E^{\circ} = F(E^{\circ}_i - E^{\circ}_j) = \Delta_{ij}I + \Delta_{ij}G^{\circ}_s$.

EPR studies

The room temperature solid state EPR spectra are typical of a tetragonal symmetry with $(dx^2-y^2)^1$ or $(d_{xy})^1$ copper (II) ion [15] for Cu-1 complex $g_{\parallel} > g_{\perp}$ and g_{\parallel} 2.189 and g_{\perp} 2.056. May be due to (i) Cu(II)-radical interaction [13,14] and (ii) similar to

Halcrow's "TPQ-on" model of CAOs enzyme [15], so also of Phalguni's molecular catalysts and (iii) due to magnetically condensed system[16]. There may be increase of positive charge on nitrogen donor atom of iminonaphthoquinone/iminonaphthoquinone valence form of ligand which reduces A_{\parallel} and increases g_{\parallel} . The solid state powder form EPR spectra of complex at 300K is presented in Fig.1.

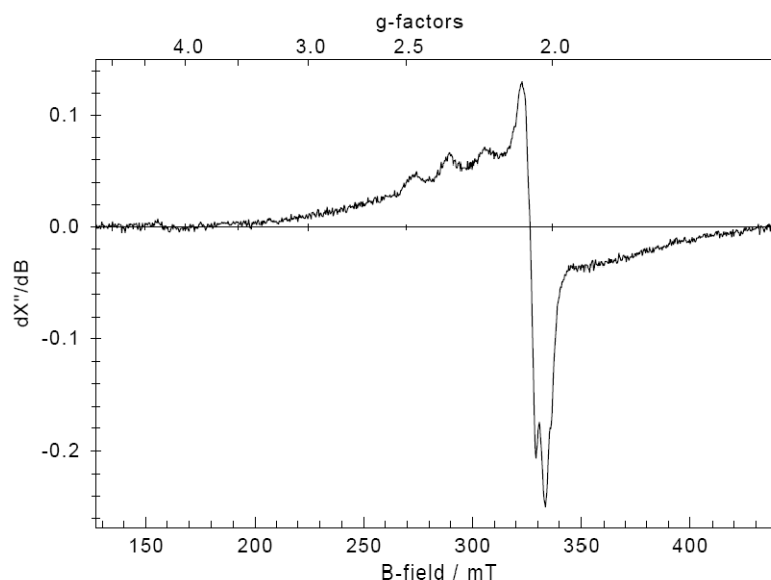


Figure 1: EPR spectra of Cu-1

However Cu-1 monomer has typical tridentate ligation of two ligands which perform hexacoordination around Cu(II) ion similar to $\text{Co}^{\text{III}}(\text{Cat-N-BQ})(\text{Cat-N-SQ})$ complex and their $g_{\parallel} > g_{\perp}$ values indicative of elongated O_h or tetragonal distortion [17]. Pierpont and coworkers had shown that the absence of spin-orbit coupling contribution to anisotropic g-factor lower than 2 in case of $\text{Cu}^{\text{II}}(\text{Phenox-SQ})_2$ complex suggesting strong ligand based spin system of open-shell coordination.

Antioxidant chemical activity

Antioxidants or antioxidant enzymes play the housekeeper's role "mopping up" free radicals before they get a chance to cause damage. Most cancer chemopreventive agents have antioxidant properties. Many of these agents become prooxidant at relatively high concentrations. This means that they may decrease or increase the cellular levels of H_2O_2 depending on their used concentration levels. It suggests that different concentrations of these agents may produce chemopreventives or chemotherapeutic effects. As scavenging of free radicals or preventing their generation or preventing oxidation of cellular oxidizable substrates is the role of antioxidant substances [18-20]. As scavenging of free radicals or preventing their generation or preventing oxidation of cellular oxidizable substrates is the role of antioxidant substances, we have to check antioxidant activity for our compounds.

Scavenging of free radicals by Cu-1 to complex with DPPH assay:

Using standard protocol with Vitamin C or ascorbic acid as a standard the radical scavenging potential of Cu-1 to complex is tested with spectrophotometric DPPH assay [21]. Ascorbate ion (Asc^-) is considered to be a terminal small molecule antioxidant and the level of this radical is a good measure of the degree of oxidative stress in biological systems and it's recommended daily allowances (RDA's) for adults are 40mg per day in Europe and 60mg per day in the US. The radical scavenging activities of Cu-1 to complexes together with Vit.C, and L-1 ligand and their reactants viz. *o*-aminophenol and lawsone are presented in Fig.2 [Scavenging of DPPH radical in terms of %inhibition vs concentration (μM)].

The decrease in absorbance at 517nm is used as a measure of antioxidant or radical scavenging activity [22] according to following hydrazyl to hydrazine conversion.

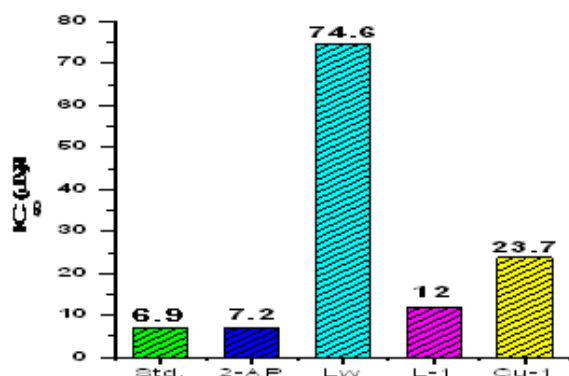
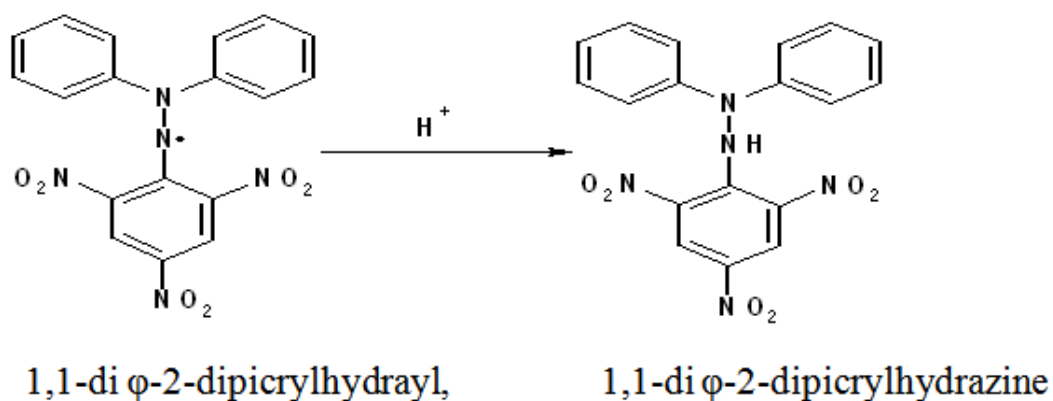


Figure 2: Scavenging of DPPH radical in terms of

Fig.2 displays bar chart of compounds IC_{50} (μM) concentrations required for % inhibition. Although IC_{50} of reactants *o*-aminophenol (AP) and lawsone (Lw) are nearly same as Std. Vit. C ($7\mu\text{M}$) and its pretty close to L-1 ($10\mu\text{M}$) ligand, % inhibition by lawsone ($\sim 24\%$) is very low. It indicates that the antioxidant activity of

lawsone (2-OH-1,4-NQ), a TPQ- analogue is raised by coupling with amine function (AP) in L-1 ligand (81%) and which is nearly same as Std. Vit. C (84%). On complexation the scavenging activity either remains same as in monomeric Cu-1 (~80%). Cu-1(80%)<L-1(81%)

Acknowledgements

NF and SK are thankful to Chemistry Department, Payame Noor University, 19395-4697 Tehran, Iran for the grant support.

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