Catechol Oxidase Activity of New Dinuclear Cu^{II}_2 and Zn^{II}_2 Complexes

Gopal Chandra Giri

Department of Chemistry, Bhairab Ganguly College, Belgharia, Kolkata-700056

e-mail : ggirigopal@gmail.com

Date of Submission: 28th July, 2015 Date of Acceptance: 3rd October, 2015

Abstract

Two new, a dinuclear copper(II) and a dinuclear zinc(II) complexes of an isoindol functionality based new dinucleating ligand, H_3hdpa (H_3hdpa = 2-({[2-hydoxyethyl]-[2-hydroxy-3-(1-oxo-1,3-dihydro-isoindol-2-yl)-propyl]-amino}-methyl)-benzoic acid) have been synthesized and characterized. In methanol, the reaction of stoichiometric amounts of $Cu(OAc)_2 \cdot H_2O$ and the ligand, H_3hdpa in the presence of NaOH at ambient temperature afforded a new dinuclear copper(II) complex, $Na_2[Cu_2(hdpa)_2] \cdot 2CH_3OH \cdot 3H_2O$ (**1**). Similarly, in methanol, the reaction of stoichiometric amounts of $Zn(OAc)_2 \cdot 2H_2O$ and H_3hdpa in the presence of NaOH yielded a new dinuclear zinc(II) complex, $Na_4[Zn_2(hdpa)_2](OAc)_2$ (**2**). Characterizations of the complexes have been performed using various analytical techniques including a single crystal X-ray structure determination. The X-ray crystal structure of complex **1** reveals that two copper(II) centers adopt a five-coordinate square pyramidal geometry with Cu---Cu separation of 2.909 Å. The DFT optimized structure of complex **2** shows that two zinc(II) centers are in a distorted trigonal bipyramidal geometry with Zn---Zn separation of 3.124 Å. ^{1}H and ^{13}C NMR spectroscopic investigations authenticate the integrity of complex **2** in solution. Further, the mass spectroscopic analyses of complexes **1** and **2** reconfirm their dimeric nature, even in solution. Variable-temperature (2-300 K) magnetic susceptibility measurements show the presence of antiferromagnetic interactions (J = -52.20 cm^{-1}) between the two copper(II) centers in complex **1**. Catechol oxidase activity of complexes **1** and **2** has been investigated in methanol medium by UV-Vis spectrophotometric technique using 3,5-di-tert-butylcatechol as model substrate. Both the two complexes are active in catalyzing the aerobic oxidation of 3,5-di-tert-butylcatechol (3,5-DTBC) to 3,5-di-tert-

butylbenzoquinone (3,5-DTBQ). DFT calculation has been performed to find the Fukui functions at the metal centers in complexes **1** and **2** to predict the possible metal centers involved in the binding process with 3,5-DTBC during the catalytic oxidation reactions.

Keywords: Dinucleating ligand, Isoindol functionality, Copper(II)/zinc(II), Magnetism, Catecholase activity

1. Introduction

Dimetallic complexes have been recognized at the active sites of many metalloenzymes[1]. In fact, enzyme models are of practical importance for the development of efficient molecular catalysts for organic reactions and of theoretical importance in elucidating the mechanisms of enzymatic reactions[2]. So, the model studies with simple dinuclear metal complexes are becoming increasingly important in understanding biological functions of the dimetallic cores[3]. Very often, in a dimetallic system, one metal ion is responsible for substrate binding while the other delivers the activated solvent nucleophile for hydrolysis. This type of dinuclear Cu_2 and Zn_2 complexes with either one loosely bound apical exogeneous ligand or coordinatively unsaturated ligand, have the potential for binding of biologically important substrates, thereby providing new reactivity patterns. In this context, dinuclear copper(II) complexes with two metal ions in close proximity have received considerable attention as structural models for catechol oxidase (CO) which catalyzes the oxidation of o-diphenol to o-quinone coupled with a $2e^-/2H^+$ reduction of O_2 to H_2O[4]. The crystal structures of CO isolated from sweet potato[5] reveals that the active site of the enzyme contains antiferromagnetically coupled EPR silent Cu_2 centers in which the Cu---Cu distance is 2.9 Å, which is a suitable distance for catechol binding[6]. In isolated CO, each copper(II) center is coordinated to three histidine nitrogen atoms and is bridged by an external hydroxo group in a trigonal pyramidal geometry. Thus, the oxygen bridged dinuclear copper(II) complexes have attracted great attention in recent years. As a result, a number of dinuclear copper(II) complexes are well documented in the literature in understanding the mode of catechol binding and the mechanistic role of the active site[7]. In contrast, the dinuclear zinc(II) complexes remain largely unexplored as synthetic models for catechol oxidase activity[8]. However, a few interesting reports on the interactions of zinc complexes with model substrates like 3,5-di-tert-butylcatechol (3,5-DTBC) or tetrachlorocatechol(TCC) are reported in the literature[9].

Recently, the coordination behavior of the various dinucleating ligands containing alkoxo and carboxylato donor groups have been much attracted[10]. The use of dinucleating carboxylate ligands as the organic linkers which are capable of binding two metal ions through direct bond formation and promoting magnetic interactions has been studied extensively[11]. We have focused here on an isoindol functionality based new unsymmetrical dinucleating ligand, H_3hdpa (Fig. 1). During N-alkylation at one half of the symmetrical precursor ligand, *N*,*N*′-Bis(2-carboxybenzomethyl)-1,3-diaminopropan-2-ol (H_3cdp)[12] with 2-iodoethanol in 1:1 molar ratio, the secondary amine and the benzoate functionality at the other half of the ligand has undergone an intramolecular cyclization reaction to produce a

new unsymmetrical ligand, H_3hdpa consisting of an isoindol functionality. The 1-oxo-1,3-dihydroisoindol ring is most likely achieved via the known mechanism that involves the activation of the secondary amine and benozoatebackbone in the presence of NaOH[13]. Recently, we have reported a heptanuclear zinc complex of a carboxylate and amide rich dinucleating ligand undergoning an intramolecular reaction between the N-alkylated amide and the benzoate functionality at the half-end of the symmetrical ligand to yield an unsymmetrical ligand with an isoindol moiety[10b]. In this paper, we report synthesis, structure, spectroscopic characterization, magnetic property and catechol oxidase activity of a new dinuclear Cu(II) and Zn(II) complexes.

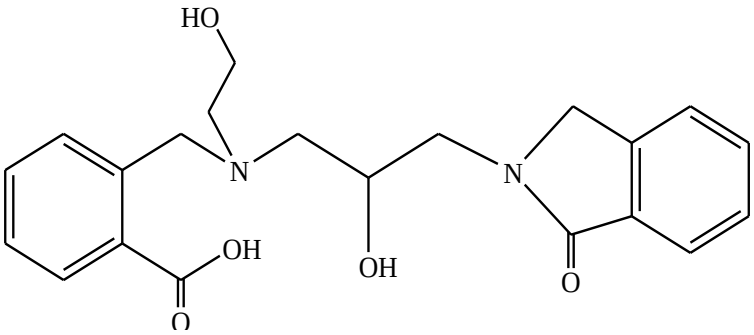

Figure1: ligand H_3hdpa.

2. Results and Discussion

Synthesis and Characterization: The unsymmetrical dinucleating ligand, H_3hdpa has been synthesized in two step reactions. The synthesis of the precursor reduced Schiff base, H_3cdp has been accomplished by the condensation of stoichiometric amounts of 2-carboxybenzaldehyde and 1,3-diamino-propan-2-ol in the presence of NaOH in methanol under refluxing conditions for 4 hr, followed by the subsequent reduction using $NaBH_4$[12]. Acidification of the resulting solution by addition of HCl to pH~5 yielded a white solid product. The product has been characterized to be a reduced Schiff base, H_3cdp·H_2O, by different analytical techniques such as elemental analysis, FTIR and NMR spectroscopy. Alkylation of the secondary amine at one half of H_3cdp with 2-iodoethanol in 1:1 molar ratio and intramolecular cyclization between the secondary amine and the benzoate functionality at the other half produced the dinucleating ligand, H_3hdpa, in good yield. The ligand, H_3hdpa is fully characterized using elemental analysis, FTIR, NMR and mass spectroscopic techniques.

The reaction of $Cu(OAc)_2·H_2O$ with the ligand H_3hdpa in 1:1 molar ratio in presence of a strong base, NaOH at pH ~ 9 in methanol afforded a green dinuclear complex, $Na_2[Cu_2(hdpa)_2]·2CH_3OH·3H_2O$ (**1**) . However, reaction of $Zn(OAc)_2·2H_2O$ with H_3hdpa in 1:1 molar ratio in presence of NaOH at pH ~ 9 in methanol yielded an off-white dinuclear complex, $Na_4[Zn_2(hdpa)_2](OAc)_2$ (**2**) . The complexes **1** and **2** are fully characterized using the techniques such as elemental analysis, FTIR, UV-vis, ^{1}H, and ^{13}C NMR spectral analyses and room temperature magnetic moment measurements. The room temperature magnetic

moment per Cu for the complex **1** is 1.71 μ_B indicating the presence of one unpaired electron in each copper ion.

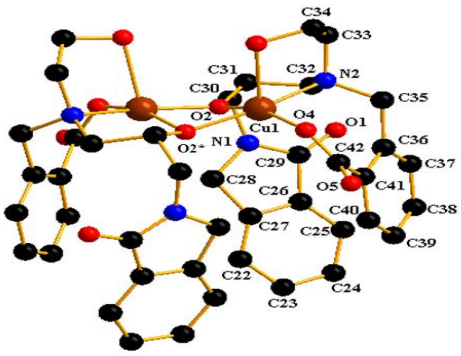

Figure 2: Crystal str. of complex 1

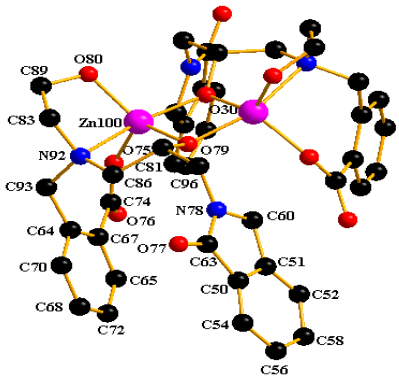

Figure 3: Crystal str. of complex 2

3. Catechol Oxidase Activity Studies and Kinetics

Catechol oxidase (CO), also known as *o*-diphenol oxidase, is a less well known member of the type-3 copper proteins[14]. The official nomenclature is 1,2-benzenediol:oxygen oxidoreductase, indicating that dioxygen is the second substrate. CO catalyzes exclusively the oxidation of catechols (i.e., *o*-diphenols) to the corresponding *o*-quinones. The catalytic oxidation reaction of catechol, in particular, 3,5-di-tert-butylcatechol (3,5-DTBC) has been widely studied as a model reaction for the catecholase activity. The substrate, 3,5-DTBC with bulky substituents on the ring has low quinone-catechol reduction potential. This makes it to be easily oxidized to corresponding *o*-quinone, 3,5-DTBQ, which is highly stable and exhibits maximum absorption at 400 nm (ε = 1900 $M^{-1}cm^{-1}$) in methanol.

Prior to the detailed kinetic investigations, we examined the catecholase activity of complexes **1** and **2** which showed catalytic oxidation activity towards the substrate 3,5-di-tert-butylcatechol. For this purpose 10^{-4} M solutions (in methanol) of these complexes were treated with a 10^{-2} M solution (~100 equiv.) of 3,5-DTBC at room temperature under aerobic conditions. The course of the reaction was followed by UV-vis spectroscopic technique. After addition of the substrate (3,5-DTBC) to solutions of catalysts **1** and **2** a gradual increase in the band that corresponds to 3,5-DTBQ was observed at 400 nm, as displayed in the UV-vis spectra.

4. Conclusion

We have described the synthesis and characterization of a new dinuclear copper(II) complex (**1**) and a new dinuclear zinc(II) complex (**2**) of an isoindol functionality based dinucleating ligand. The X-ray structural and spectroscopic investigations confirmed the dimetallic nature of complexes **1** and **2** both in solid state as well as in solution. The complex **1** shows an antiferromagnetic interaction (J = -52.20 cm^{-1}) between the two copper centers identified from variable temperature (2-300 K) magnetic susceptibility measurements. The complexes maintain the metal-metal separation falling in the range of 2.9 to 3.1 Å which is optimum

cooperativity between the two metal centers for mimicking the structural and functional models to the active site of catechol oxidase. In a simulated metal-metal distance and NO_5 coordination environment, both the complexes show catecholase like activity. Density Functional Theory (DFT) calculations strongly suggest that during the catalytic oxidation reaction of 3,5-DTBC, the binding of substrate with the dinuclear metal complexes **1** and **2** occurs more possibly through the involvement of two metal centers. The present investigations will positively provide valuable insights into the biologically relevant coordination chemistry of copper and zinc.

References:

1. (a) D. E.Fenton, H. Okawa, *PerspectiVes on Bioinorganic Chemistry*; JAI Press: London, UK, **2**, 81-137 (1993**)**. (b) K. D. Karlin, *Science* **261**, 701-708 (1993**)**.
2. (a) R. Breslow, *Acc. Chem. Res.* **24**, 317-324 (1991). (b) A. J. Kirby, *Angew. Chem. Int. Ed. Engl.* **35**, 707-724 (1996).
3. (a) D. E. Wilcox, *Chem. Rev.* **96**, 2435-2458 (1996**)**. (b) N. Strater, W. N. Lipscomb. T. Klabunde, B. Krebs, *Angew. Chem., Int. Ed. Engl.* **35**, 2024-2055 (1996).
4. (a) P. K.Nanda, V. Bertolasi, G. Aromi, D. Ray, *Polyhedron* **28**, 987-993 (2009). (b) J. E.Bol, W. L. Driessen, J. Reedijk, *J. Chem. Soc., Chem. Commun.*, 1365-1366 (1995) (c) S. Torelli, C. Belle, I. Gautier-Luneau, J. L. Pierre, *Inorg. Chem.* **39**, 3526-3536 (2000**)**. (d) Y. C. Chou, S. F. Huang, R. Koner, G. H. Lee, Y. Wang, S. Mohanta, H. Wei, *Inorg. Chem.* **43**, 2759-2761 (2004).
5. T. Klabunde, C. Eicken, J. C. Sacchettini, B. Krebs, *Nat. Struct. Biol. 5*, 1084-1090 (1998)**.**
6. (a) A. Sanchez-Ferrer, J. N. Rodriguez-Lopez, F. Garcia-Canovas, F. Garciacarmona, *Biochim. Biophys. Acta*. **1247**, 1-11 (1995 (b) C. Eicken, B, Krebs, J. C. Sacchettini, *Curr. Opin. Struct. Biol.* **9.**, 677-683 (1999). (c) C. Gerdemann, C. Eicken, B. Krebs, *Acc. Chem. Res.* **35**, 183-191 (2002).
7. (a) A. Biswas, L. K. Das, M. G. B. Drew, C. Diaz, A. Ghosh, *Inorg. Chem.* **51**, 10111-10121 (2012). (b) J. Mukherjee, R. Mukherjee, *Inorg. Chim. Acta* **337.**, 429-438 (2000). (b) K. S. Banu, T. Chattopadhyay, A. Banerjee, S. Bhattacharya, E. Suresh, M, Nethaji, E. Zangrando, D. Das, *Inorg. Chem.* **47**, 7083-7093 (2008).
8. A. Guha, T. Chattopadhyay, N. D. Paul, M. Mukherjee, Goswami, T. K. Mondal, E. Zangrando, D. Das, *Inorg. Chem.* **51**, 8750-8759 (2012).
9. (a) M. E. Bodini, G. Copia, R. Robinson, D.T. Sawyer, *Inorg. Chem.* **22**, 126-129 (1983). (b) M. Ruf, B. C. Noll, M. D. Groner, G. T. Yee, C. G. Pierpont, *Inorg. Chem.* **36**, 4860-4865 (1997).
10. (a) M. Bera, A. B. S. Curtiss, G. T. Musie, D. R. Powell, *Inorg. Chem. Commun*. **11.**, 1033-1036 (2008). (b) M. Bera, G. T. Musie, D. R. Powell, *Inorg. Chem.* **48**, 4625-4627 (2009). (c) M. Bera, G. T. Musie, D. R. Powell, *Inorg. Chem. Commun*. **13.**, 1029-1031 (2010). (d) A. Patra, T. K. Sen, R. Bhattacharyya, S. K. Mandal, M. Bera, *RSC Adv*. **2**, 1774-1777 (2012). (e) M. Bera, A. B. S. Curtiss, G. T. Musie, D. R. Powell, *Inorg. Chem.* **51**, 12093-12101 (2012).
11. (a) R. A. Joy, H. Arman, S. Xiang, G. T. Musie, *Inorg. Chim. Acta* **394**, 220-228 (2013). (b) C. H. Weng, S. C. Cheng, H. M. Wei, H. H. Wei, C. J. Lee, *Inorg. Chim. Acta* **359**, 2029-2040 (2006). (c) A. Mukherjee, M. K. Saha, M. Nethaji, A. R. Chakravarty, *New. J. Chem*. **29**, 596-

603 (2005). (d) M. Kodera, N. Terasako, T. Kita, Y. Tachi, K. Kano, M. Yamazaki, M. Koikawa, T. Tokii, *Inorg. Chem*. **36**, 3861-3868 (1997).

12. A. B. S. Curtiss, M. Bera, G. T. Musie, D. R. Powell, *Dalton Trans*. **20**, 2717-2724 (2008).
13. (a) M. Kunishima, K. Hioki, T. Moriya, J. Morita, T. Ikuta, S. Tani, *Angew. Chem., Int. Ed.* **45**, 1252-1255 (2006). (b) M. Pohmakotr, N. Yotapan, P. Tuchinda, C. Kuhakarn, V. Reutrakul, *Tetrahedron* **63**, 4328-4337 (2007). (c) J. M. Concellon, E. Bardales, *Org. Lett.* **5**, 4783-4785 (2003).
14. E. I. Solomon, U. M. Sundaram, T. E. Machonkin, *Chem. Rev*. **96**, 2563-2606 (1996).