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Novel Diphenoxo-Bridged Dinuclear Zinc Complexes: Generation of Phenoxyl-Radical Species and Nuclease Activity

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Tridentate ligand PhimpH having N_2O donors binds to zinc(II) after deprotonation, affording novel diphenoxo-bridged dinuclear zinc complexes $[Zn_2(Phimp)_2(ClO_4)_2]$ (**1**) and $[Zn_2(Phimp)_2(Cl)_2]\cdot CH_2Cl_2$ ($\mathbf{2}\cdot CH_2Cl_2$). The molecular structure of $\mathbf{2}\cdot CH_2Cl_2$ revealed some unique structural features. The phenoxyl-radical complexes were generated at room temperature, and these radical complexes exhibited nuclease activity with pBR322 DNA.

Copper and zinc both possess stable 2+ oxidation states, and in several cases, analogous complexes of zinc help to interpret the magnetic, spectroscopic, redox, and structural properties of copper complexes.[1] For example, in the structural and functional modeling of the active site of galactose oxidase enzyme, analogous zinc complexes were synthesized and characterized and their reactivities were investigated.[2-5] Relevant with copper chemistry, the zinc complexes derived from ligand-containing phenolato donor(s) could undergo ligand-centered oxidation, affording phenoxyl-radical complexes.

Investigation of the literature revealed that phenoxyl-radical complexes were stabilized by the −SR group at the ortho position to the phenolato function and/or bulky alkyl group in the phenyl ring bearing a phenolato donor.[2-5] To the best of our knowledge, there is only a single report of stable phenoxyl-radical complexes by Reedijk and co-workers.[6] In the report, ligands were devoid of radical-stabilizing substituents. Recently, we reported[7] copper complexes and corresponding phenoxyl-radical complexes derived from PhimpH (Scheme S1 in the Supporting Information, SI). The phenoxyl-radical complexes were stable only at low temperature (0−5 °C) and were characterized by UV−vis spectral studies. This work stems from our interest in the chemistry of zinc complexes coordinated to phenolato ligand(s). Zinc complexes derived from ligand PhimpH were synthesized, and phenoxyl-radical complexes were generated in a CH_3CN solution by the addition of ceric ammonium nitrate (CAN) at room temperature (25 °C). We observed the rapid formation of a green solution after the addition of CAN in CH_3CN and the disappearance of the green color thereafter. The rate of disappearance of the green color was found to be slower compared to that in the case of copper complexes derived from the same ligand.[7] PhimpH does not contain any −SR or bulky alkyl group. This prompted us to determine the structure of the resultant complexes. The structure determination revealed a novel diphenoxo-bridged dinuclear zinc complex, whereas in the case of copper, we ended up with different types of complexes.[7]

Zinc is biologically relevant and a less toxic metal.[8] Because of their strong Lewis acidity and rapid ligand-exchange property, mononuclear and polynuclear zinc complexes were studied for the development of artificial nucleases.[6] It is well-known in the literature that in situ generated radicals are the key species for nuclease activity by chemical nucleases.[9] Phenoxyl-radical complexes and/or reactive radical species in solution produced during decomposition of radical complexes[10] could be utilized for nuclease

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(1) Silvestri, A.; Barone, G.; Ruisi, G.; Anselmo, D.; Riela, S.; Liveri, V. T. *J. Inorg. Biochem.* **2007**, *101*, 841.

(2) (a) Sokolowski, A.; Muller, J.; Weyhermuller, T.; Schnepf, R.; Hildenbrandt, P.; Hildenbrand, K.; Bothe, E.; Wieghardt, K. *J. Am. Chem. Soc.* **1997**, *119*, 8889. (b) Bill, E.; Muller, J.; Wehermuller, J.; Wieghardt, K. *Inorg. Chem.* **1999**, *38*, 5795. (c) Chaudhuri, P.; Hess, M.; Muller, J.; Hildenbrand, K.; Bill, E.; Weyhermuller, T.; Wieghardt, K. *J. Am. Chem. Soc.* **1999**, *121*, 9599.

(3) (a) Itoh, S.; Taki, M.; Kumei, H.; Takayama, S.; Nagatomo, S.; Kitagawa, T.; Sakurada, N.; Arakawa, R.; Fukuzumi, S. *Inorg. Chem.* **2000**, *39*, 3708. (b) Taki, M.; Kumei, H.; Itoh, S.; Fukuzumi, S. *J. Inorg. Biochem.* **2000**, *78*, 1. (c) Itoh, S.; Taki, M.; Takayam, S.; Nagatomo, S.; Kitagawa, T.; Sakurada, N.; Arakawa, R.; Fukuzumi, S. *Angew. Chem., Int. Ed.* **1999**, *38*, 2774. (d) Shimazaki, Y.; Yajima, T.; Shiraiwa, T.; Yamauchi, O. *Inorg. Chim. Acta* **2009**, *362*, 2467.

(4) (a) Taki, M.; Hattori, H.; Osako, T.; Nagatomo, S.; Shiro, M.; Kitagawa, T.; Itoh, S. *Inorg. Chim. Acta* **2004**, *357*, 3369. (b) Taki, M.; Kumei, H.; Nagatomo, S.; Kitagawa, T.; Itoh, S.; Fukuzumi, S. *Inorg. Chim. Acta* **2000**, *300*, 622.

(5) (a) Mukherjee, A.; Lloret, F.; Mukherjee, R. *Inorg. Chem.* **2008**, *47*, 4471. (b) Orio, M.; Philouze, C.; Tarjayes, O.; Neese, F.; Thomas, F. *Inorg. Chem.* **2010**, *49*, 646. (c) Anjos, A. D.; Bartoluzzi, A. J.; Osorio, R. E. H. M. B.; Peralta, R. A.; Friedermann, G. R.; Mangrich, A. S.; Neves, A. *Inorg. Chem.. Commun.* **2005**, *8*, 249. (d) Zurita, D.; Scheer, C.; Pierre, J.-L.; Aman, E. S. *J. Chem. Soc., Dalton Trans.* **1996**, 4331.

(6) Maheswari, P. U.; Barends, S.; Yoman, S. O.; Hoog, P.; Casellas, H.; Teat, S. J.; Massera, C.; Lutz, M.; Spek, A. L.; Wezel, G. P.; Gamez, P.; Reedijk, J. *Chem.—Eur. J.* **2007**, *13*, 5213.

(7) Ghosh, K.; Kumar, P.; Tyagi, N.; Singh, U. P.; Aggarwal, V.; Baratto, M. C. *Eur. J. Med. Chem.* **2010**, *45*, 0000.

(8) Li, J.-H.; Wang, J.-T.; Zhang, L.-Y.; Chen, Z.-N.; Mao, Z.-W.; Ji, L.-N. *Inorg. Chim. Acta* **2009**, *362*, 1918.

(9) Pogozelski, W. K.; Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089.

(10) Altwicker, E. R. *Chem. Rev.* **1967**, *67*, 475.

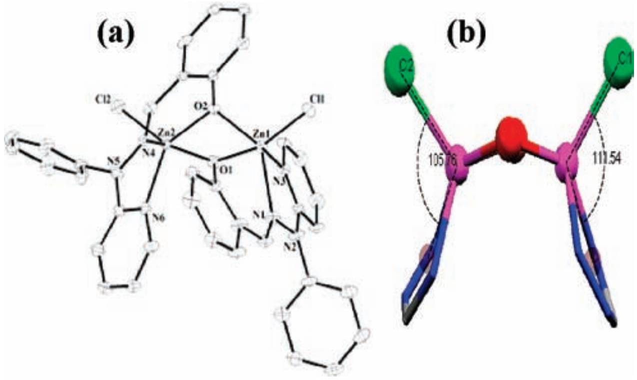

Figure 1. (a) ORTEP diagram of complex $\mathbf{2}\cdot CH_2Cl_2$ showing 50% thermal probability ellipsoids. Selected bond lengths (Å): Zn1−O1 2.019(3), Zn2−O1 2.039(3), Zn1−O2 2.041(3), Zn2−O2 2.024(3), Zn1−Cl1 2.2269(10), Zn2−Cl2 2.2535(12), Cl1−Cl2 6.206, O1−O2 2.505, Zn1−Zn2 3.0960(10). (b) Cis configuration along the Zn−Zn axis in $\mathbf{2}\cdot CH_2Cl_2$.

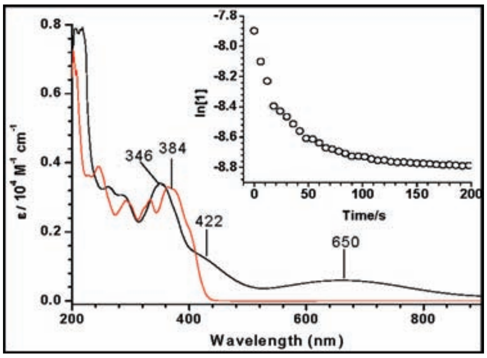

Figure 2. UV−vis spectral change observed upon oxidation of complex **1** (10×10^{-5} M) (red line) with CAN (20×10^{-5} M) (black line) in CH_3CN at 25 °C. Inset: plot of ln [**1**] vs time derived from a time course of the absorption change at 650 nm due to decomposition of the phenoxyl-radical complex of **1** at 25 °C.

activity studies. This prompted us to study the DNA interaction and nuclease activities of these zinc complexes and their phenoxyl-radical complexes. Hence, we report here two novel zinc complexes, $[Zn_2(Phimp)_2(ClO_4)_2]$ (**1**) and $[Zn_2(Phimp)_2(Cl)_2]\cdot CH_2Cl_2$ ($\mathbf{2}\cdot CH_2Cl_2$), derived from ligand PhimpH. The molecular structure of $\mathbf{2}\cdot CH_2Cl_2$ was determined by X-ray crystallography. The phenoxyl-radical complexes were generated in a CH_3CN solution and characterized by UV−vis and electron paramagnetic resonance (EPR) spectral studies. We investigated the DNA binding properties of the above two complexes. The nuclease activity of **1** and **2** and in situ generated phenoxyl-radical complexes was also examined.

Reaction of the deprotonated PhimpH with $Zn(ClO_4)_2\cdot 6H_2O$ and $ZnCl_2\cdot 2H_2O$ afforded **1** and $\mathbf{2}\cdot CH_2Cl_2$, respectively. Details of the synthetic procedures and spectroscopic characterization are described in the SI. Splitting was observed in IR spectra of **1** (Figure S1 in the SI) possibly due to a zinc-coordinated perchlorate ion.[7] However, solution conductivity measurement of **1** indicated dissociation of the perchlorate ion. All attempts to obtain well-resolved ^1H NMR spectra of **1** were unsuccessful, and we ended up with broad signals in solvents like MeOD, $CDCl_3$, and CD_3CN (see the SI). Complex **1** was converted into **2** with the help of Et_4NCl. Hence, these data indicated similar coordination modes for perchlorate and chloride ions to the zinc center (vide infra) in the solid state.

The molecular structure of $\mathbf{2}\cdot CH_2Cl_2$ was determined by X-ray diffraction studies and is displayed in Figure 1a. Complex **2** adopted a dinuclear structure that consisted of two pentacoordinated zinc atoms bridged by two phenolato moieties provided by two $Phimp^-$. The remaining parts of the ligands (one imine nitrogen atom and one pyridine nitrogen atom for each ligand) were bound to zinc centers. A structural index parameter calculation[11] for $\mathbf{2}\cdot CH_2Cl_2$ showed τ values of 0.026 and 0.230 for zinc centers. These data supported a distorted square-pyramidal geometry around the metal centers; however, the Zn2 center was more distorted than the Zn1 center (details are given in the SI). The equatorial plane was generated by two bridged phenolate oxygen atoms (O1 and O2), one imine nitrogen atom (N1), and one pyridine nitrogen atom (N3), whereas the axial position is occupied by a chloride anion (Cl1). Zn1 is ~0.76 Å away from the mean plane constituted by O1, O2, N1, and N3; similarly, Zn2 is ~0.73 Å away from the mean plane generated by O1, O2, N4, and N6.

Interestingly, the two chloride ions (Cl1 and Cl2) are on the same side of the Zn−Zn axis; consequently, the ligands ($Phimp^-$) occupy the other side, giving rise to an overall unique cis configuration (Figure 1b). We have found the presence of a noncovalent interaction between the chloride ions and the dichloromethane molecule and a zigzag structure in the packing diagram of $\mathbf{2}\cdot CH_2Cl_2$ (shown in the SI). Cis disposition of the chloride ions along the Zn−Zn axis is different from the structure reported by Reedijk and coworkers[6] and Chen et al.[12] These two cis-disposed Zn1−Cl1 and Zn2−Cl2 distances in $\mathbf{2}\cdot CH_2Cl_2$ are 2.23 and 2.25 Å, respectively, and are consistent with the reported data.[6,12]

All of the Zn−N distances are consistent with the literature;[6,4a] however, the Zn−Zn distance (3.09 Å) is in the lower range of the reported values (2.94−3.24 Å).[5a,6,12,13] There are two Zn−O distances found for the Zn_2O_2 moiety. The difference in the two distances was found to be 0.02 Å for $\mathbf{2}\cdot CH_2Cl_2$. Investigation of the literature gave rise to differences of 0.04−0.18 Å for similar diphenolato-bridged dinuclear zinc complexes.[4a,5a,6,12,13] Hence, $\mathbf{2}\cdot CH_2Cl_2$ contains the most symmetrical Zn_2O_2 moiety among all of the reports available in the literature.

The addition of 2 equivalents of CAN in the acetonitrile solution of complexes **1** and **2** afforded three new absorption bands due to generated phenoxyl-radical complexes (shown in Figures 2 and S9 in the SI for **1** and **2**, respectively). Upon the addition of CAN, the charge-transfer band of the complexes readily disappeared with the concomitant appearance of three new characteristic absorption bands at 346, 422, and 650 nm for **1** and 340, 420, and 630 nm for **2**, which clearly indicated the formation of phenoxyl-radical complexes.[2−5] The intensity of these bands increased as a result of the addition of 3 and 4 equivalents of CAN with generation of a peak near 420 nm. The further addition of CAN did not afford any change of the UV−vis spectra (Figures S10 and S11 in the SI).

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(11) Addison, A. W.; Rao, T. N.; Reedijk, J.; Rijn, J. V.; Verschoor, G. C. *J. Chem. Soc., Dalton Trans.* **1984**, 1349.

(12) Chen, C.-T.; Chang, W.-K.; Sheu, S.-C.; Lee, G.-H.; Ho, T.-I.; Wang, Y. *J. Chem. Soc., Dalton Trans.* **1991**, 1569.

(13) Silvernail, C. M.; Yao, L. J.; Hill, L. M. R.; Hillmyer, M. A.; Tolman, W. B. *Inorg. Chem.* **2007**, *46*, 6565.

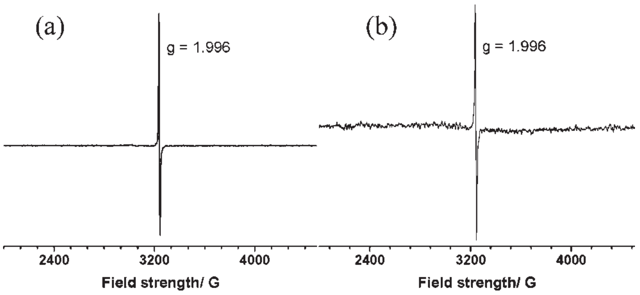

Figure 3. X-band EPR spectra of phenoxyl-radical complexes derived from **1** (a) and **2** (b) in CH_3CN at 120 K.

The X-band EPR spectra of phenoxyl-radical complexes derived from **1** and **2** in CH_3CN at 120 K are shown in Figure 3. The EPR spectra exhibited an isotropic sharp signal at $g = 1.996$ for radical complexes derived from **1** and **2** and were typical for phenoxyl-radical ($S = {}^1/_2$) species.[5] This single sharp signal indicated the absence of coupling due to a benzylic proton, an imine nitrogen, or a ^{67}Zn isotope ($I = {}^5/_2$).[2a]

Phenoxyl-radical complexes were fairly stable at room temperature, and the decomposition reactions of **1** and **2** were monitored by a time-dependent study at a particular λ value (650 and 630 nm for **1** and **2**, respectively; Figures 2, inset, and S9 in the SI). Investigation of the data indicates that decomposition of radical complexes probably followed second-order kinetics.[4a] The stability of phenoxyl-radical complexes generated from **1** was greater than that of **2**. This was also supported by a red shift of the characteristic peak above 600 nm on going from **2** to **1**.[4a] Purging of oxygen through a solution containing radical complexes did not enhance the rate of decomposition and, hence, we speculate that the solvent may have a major role in the decomposition process.[4a,10]

DNA interaction of **1** and **2** was investigated by UV−vis, fluorescence, and circular dichroism spectral studies. Absorption binding constants (K_b) derived from UV−vis spectral data, Stern−Volmer constants (K_{sv}) determined by a fluorescence quenching experiment, and circular dichroism spectral data support external or surface binding of **1** and **2** with calf thymus DNA (shown in the SI).

We extended our DNA interaction studies by examining the nuclease activity of the complexes as well as the phenoxyl-radical complexes. Upon investigation of the DNA gel electrophoresis data (Figures 4 and S18 in the SI), the following important observations were made. First, complexes **1** and **2** did not participate in hydrolytic and/or oxidative nuclease activity (lanes 3 and 5 in Figure 4). Second, both the complexes exhibited negligible activity in the presence of H_2O_2 and 2-mercaptoethanol (Figure S18 in the SI).

Third, and most interestingly, when phenoxyl-radical complexes were generated in situ in a solution containing pBR322 DNA, nuclease activity was observed. No such activity was found for pBR322 DNA in the presence of CAN (lane 2, Figure 4). In the case of copper complexes, nuclease activity was proportional to the incubation time;[7] however; our result showed that the nuclease activity by radical complexes was independent of the incubation time. This may be due to decomposition of radical complexes within 10 min. These data clearly indicated that phenoxyl-radical generation was very important and a key step for nuclease activity. Hence, zinc-radical complexes and/or some other radical species generated during the decomposition process[10] could be responsible for DNA cleavage activity. We tried to investigate the mechanism of nuclease activity in the presence of radical scavengers.[14] None of the scavengers, excluding dimethyl sulfoxide, showed inhibition of the nuclease activity (Figure S19 in the SI). Insights into the details of mechanistic studies are in progress.

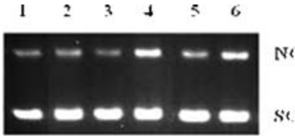

Figure 4. Gel electrophoresis separations showing the in situ cleavage of supercoiled pBR322 DNA (100 ng) by complexes **1** and **2** (100 μM) in the presence of CAN (200 μM). 5% acetonitrile was used as the medium and incubated at 37 °C for 1.5 h: lane 1, DNA; lane 2, DNA + CAN; lane 3, DNA + **1**; lane 4, DNA + **1** + CAN; lane 5, DNA + **2**; lane 6, DNA + **2** + CAN.

In conclusion, two novel diphenoxo-bridged dinuclear zinc complexes, **1** and $\mathbf{2}\cdot CH_2Cl_2$, were synthesized, and the molecular structure of $\mathbf{2}\cdot CH_2Cl_2$ was determined. The structure afforded a unique cis orientation of Cl^- and $Phimp^-$ along the Zn−Zn axis, giving rise to a butterfly shape of the molecule. A highly symmetrical Zn_2O_2 core afforded a very short Zn−Zn distance of 3.09 Å. Phenoxyl-radical complexes were generated at room temperature and characterized by UV−vis and EPR spectral studies. Hence, these are novel examples of dinuclear zinc complexes where the phenoxyl radical was stabilized without the presence of a radical stabilizing group. Phenoxyl-radical complexes exhibited nuclease activity. Reedijk and co-workers studied the DNA cleavage activity of the complexes without adding CAN because the radicals were already generated in the solid state. To the best of our knowledge, this is the first example of nuclease activity studies on phenoxyl-radical complexes generated by CAN. Work is in progress to gain better insight into the stability of the complexes, oxidation chemistry,[3b] and mechanism of nuclease activity.

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Supporting Information Available: Crystallographic data in CIF format, experimental details, spectroscopic characterization, and gel electrophoresis. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) Chen, J.; Wang, X.; Shao, Y.; Zhu, J.; Zhu, Y.; Li, Y.; Xu, Q.; Guo, Z. *Inorg. Chem.* **2007**, *46*, 3306.